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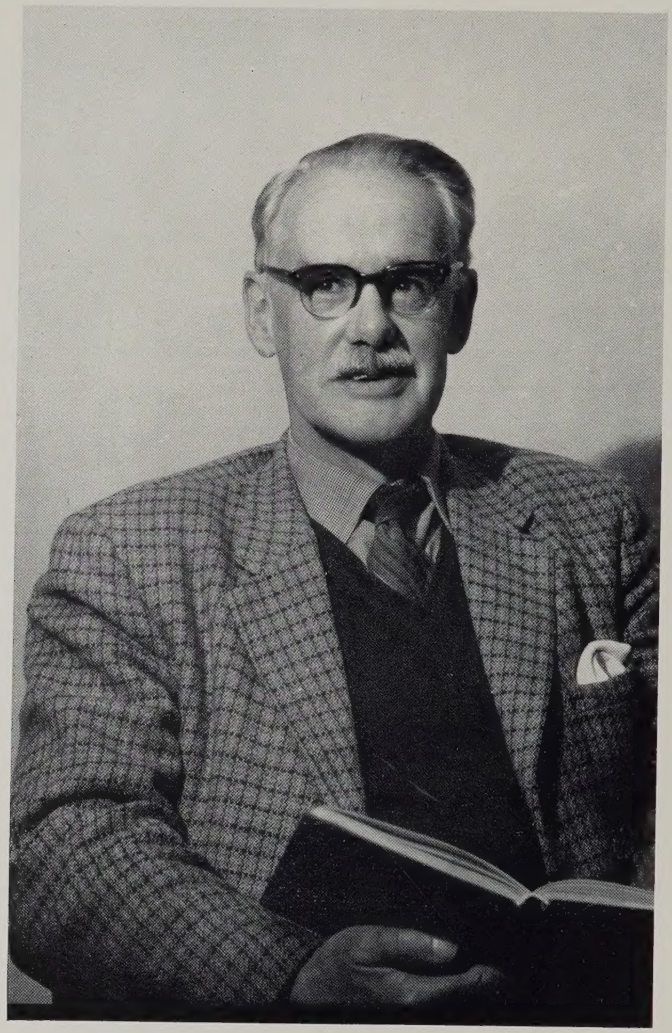

DR H. F. BARNES

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(*Facing p.* 205)

Ann. appl. Biol. **48** (2), 205–206.

OBITUARY

Dr H. F. BARNES

Horace Francis Barnes died suddenly on 4 February 1960. He joined the Association in 1927, served as a Member of Council from 1936 to 1938, as Zoological Secretary from 1939 to 1944 and as Treasurer from 1947 until his death. Thus for 22 years he helped in many ways to guide the Association, and for the last 13, as the Officer with the longest service, he played a leading part in developing its post-war expansion. He was a shrewd and meticulous Treasurer, spending a considerable amount of his time on Association activities but never spending its money unnecessarily. The healthy state of our finances today is a tribute to the care with which he has husbanded our resources.

Barnes had a characteristic charm and kindness that endeared him to all who were privileged to know him, and few people have engendered such widespread esteem and respect. He was somewhat spartan in outlook, yet quietly enjoyed his pipe, tankard and other simple pleasures. His sense of responsibility and loyalty was outstanding and he allowed nothing to interfere with his work or deflect him from what he considered to be the path of duty. Integrity and industry are perhaps the two words that best describe his character.

Born on 23 November 1902, the son of the Chaplain of Bedford School, Barnes was later educated there and at Queen's College, Oxford, graduating in the School of Zoology and Comparative Anatomy in 1924. He was awarded the Ph.D. (London) in 1928 and M.A. (Oxon) in 1932.

From 1924 to 1927 he worked as a Ministry of Agriculture Research Scholar on Cecidomyidae affecting cereals, first at the British Museum, then at the Agricultural College, Wye, for 2 years, and finally at the New York State Museum. Thus he early and soundly laid the foundations on which he built his life's work, for he will be remembered longest as the world's foremost expert on the gall midges.

In 1927 he was appointed to the staff of Rothamsted Experimental Station, Harpenden, where he spent the remainder of his life. That his work there was of outstanding merit was recognized by his promotion to the grade of Senior Principal Scientific Officer. Most of his work was on the taxonomy and biology of cecids, as the list of his publications so amply demonstrates, and he crowned it by publishing seven volumes on the *Gall Midges of Economic Importance*, which will long remain the world 'source book' on these insects. His thorough and detailed study of the ecology of slugs in gardens and allotments also made him an accepted authority on these animals.

Barnes, a lucid and amusing lecturer, was always interested in education and, though never a teacher, he served as external examiner in entomology or agricultural zoology for the Universities of London, Reading, Travancore and Agra

(India), and Seale Hayne Agricultural College. In 1952 he became editor of *The Bedfordshire Naturalist*, after having served for 20 years as an abstractor for the *Journal of Animal Ecology* and a reviewer for *Science Progress* for 11 years.

He travelled abroad several times on official visits to research institutes and scientific congresses and was honoured by being elected a Fellow of the Entomological Society of America in 1957 and a Corresponding Member of the Entomological Society of Finland in 1949.

We shall remember Frank Barnes with gratitude for his scientific work and his inestimable services to the Association. We also remember and extend our sympathy to Mrs Barnes, who did so much to help him, and often accompanied him to our meetings, and to his daughter and two sons.

L. BROADBENT

LAST, F. T. (1960). *Ann. appl. Biol.* 48 (2), 207–229.

EFFECT OF CULTURAL TREATMENTS ON THE INCIDENCE OF *STRIGA HERMONTHICA* (DEL.) BENTH. AND YIELDS OF SORGHUM IN THE SUDAN: FIELD EXPERIMENTS 1957/8

BY F. T. LAST*

Gezira Research Station, Wad Medani, Sudan

(With 7 Text-figures)

Trap cropping, nitrogenous fertilizers, selective weed-killer sprays (2:4-D) and sorghum varieties affected the incidence of *Striga hermonthica*.

S. hermonthica developed more on the variety Debekri than Feterita. Usually, applying increasing amounts of nitrogen (sulphate of ammonia or urea) progressively decreased the incidence of *S. hermonthica* on Feterita, Dwarf White Milo and Wad Fahl. Early applications were more effective than late applications. Nitrogen given to Debekri never significantly decreased the amount of *S. hermonthica* and often increased it.

In an experiment on very infertile and heavily infested soil, nitrogen increased the amounts of *S. hermonthica* on all varieties but less on Feterita than Debekri. The weights of *S. hermonthica* on Feterita reached a maximum with 40 lb. N/acre but were still increasing after adding 80 lb. N to Debekri.

Weed-killer sprays affected *S. hermonthica* on Feterita and Debekri equally. They delayed the appearance of its aerial stems and usually decreased their subsequent growth. Spraying with 1·6 lb. 2:4-D/acre was usually more effective than with 0·8 lb. and sometimes spraying 3 weeks after sowing was better than after 2 weeks.

Trap cropping with Sudan grass (*Sorghum sudanense*) for 5 weeks, but not for 3, significantly decreased the incidence of *S. hermonthica* in succeeding sorghum crops and sometimes increased grain yields.

Phosphorus and potassium affected neither the incidence of *S. hermonthica* nor sorghum yields. On lightly infested soil where *S. hermonthica* was not damaging, 80 lb. N/acre trebled grain yields (from 1140 to 3530 lb./acre). As infestations increased in severity, the absolute gains decreased but the relative effects were greater. On crops infected early and severely, yields increased by 820% from 180 to 1505 lb./acre. Nitrogen applied at sowing and after 3 weeks gave larger increases than later applications.

Yield increases caused by 2:4-D sprays are associated with the delayed emergence of aerial stems of *S. hermonthica*. Where infection developed rapidly and the mean levels of infection were at least 20% within 50 days of sowing, sprays increased both grain and straw yields; where infection developed slowly, only grain yields were increased. Although 1·6 lb. of 2:4-D/acre decreased *S. hermonthica* more than 0·8 lb., they affected yields equally as did spraying 2 or 3 weeks after sowing.

0·8 lb. of 2:4-D/acre and 80 lb. N/acre when applied separately to severely infected crops increased grain yields from 38 lb. in the untreated plots to only 163 and 588 lb./acre respectively, whereas applied together they gave 2170 lb./acre. In the same experiment nitrogen increased the yields of the less heavily infected Feterita more than those of Debekri. 2:4-D affected both varieties similarly.

* Now returned to Rothamsted Experimental Station, Harpenden, Herts., after being seconded to the Sudan Government.

INTRODUCTION

Many species of the genus *Striga* Lour. (Scrophulariaceae) parasitize roots of important tropical crops; for example, sorghum, maize and sugar cane. Infestations of *S. hermonthica*, witchweed, become increasingly severe when land is continuously cropped with sorghum, which eventually becomes unprofitable to grow (Andrews, 1945; Portères, 1952).

The seeds of *S. hermonthica*, like those of other *Striga* spp., germinate readily only when stimulated by root exudates from a limited range of host and non-host plants (McGrath *et al.* 1957)—an effect that can partly be reproduced with D-xyloketose (Brown *et al.* 1949). After germination, *Striga* seedlings send out haustoria which invade host roots and penetrate to the xylem (Uttaman, 1950). Subsequently aerial shoots may develop, with chlorophyll, as in the semi-parasitic *S. hermonthica*, or without as in the wholly parasitic *S. gesnerioides* (Willd.) Vatke (Syn. *S. orobanchoides* (R.Br.) Benth.) (Andrews, 1956).

Attempts to control *S. hermonthica* in the Sudan have centred upon the use of hormone-type weed-killers (Wilson-Jones, 1952, 1953*a*, 1956), although Crowther (1942) indicated that it may be decreased by applying nitrogenous fertilizer. In South Africa and Rhodesia, Saunders (1933) and Timson (1945) successfully used trap crops against *S. asiatica* and in Tanganyika efforts are being made to breed 'resistant' varieties (Doggett, 1953, 1958). To assess the relative values of these four possible methods of control, viz. (*a*) trap crops, (*b*) sorghum varieties, (*c*) selective weed-killers, and (*d*) fertilizers, series of field experiments were done in 1957 and 1958.

EXPERIMENTAL DETAILS

Experiments were done on alkaline clay soils at Zeidab (lat. 17° 26′ N.; long. 33° 54′ E.) *c.* 175 miles north-east of Khartoum, and at the Gezira Research Station, Wad Medani (lat. 14° 24′ N.; long. 33° 29′ E.) *c.* 100 miles south of Khartoum. The experimental sites had grown sorghum continuously for many years, without fertilizer, and became naturally infested with *S. hermonthica*.

At Wad Medani sorghum was sown on ridges in July, during the rains (15·0 in./year), irrigated when necessary and harvested in November (Table 1). Although in the Gezira rotation it usually follows cotton, in the present series of experiments

TABLE 1. *Experimental details*

		Sorghum		Sudan grass	
I. Exps. done in 1957	Site	Planted	Harvested	Planted	Harvested
1	Wad Medani	21 July	18–25 Nov.	6, 20 June	10–12 July
2	Zeidab	1 Sept.	20–6 Dec.	24 July, 7 Aug.	28 Aug.
3	Wad Medani	23 July	23–6 Nov.	—	—
4	Zeidab	1 Sept.	20–6 Dec.	—	—
II. Exps. done in 1958					
5	Wad Medani	23 July	8 Nov.	—	—
6	Wad Medani	13–14 July	2 Nov.	—	—
7	Zeidab	1 Sept.	20–5 Dec.	—	—

sorghum was planted on land known from observing the previous season's crop to be heavily infested with *S. hermonthica*.

At Zeidab, where the soil is more permeable, sorghum was sown on the flat in September and harvested in November and December.

Plots varying from $\frac{1}{24}$ to $\frac{1}{20}$ acre and watered independently were planted with seeds dusted with a fungicide/insecticide mixture containing 25% tetramethyl thiuram disulphide and 20% γ-B.H.C. at the rate of 1:450 w/w of seed (Tarr, 1954). Groups of about eight seeds were put in holes 50 cm. apart, in rows 80 cm. apart—a seed rate of about 8 lb./acre.

Varying factorial combinations of four series of treatments—sorghum varieties, weed-killer sprays, fertilizers and trap crops, were tested in seven experiments as follows.

Experiments done in 1957

Similar experiments were done at Wad Medani and Zeidab using the locally popular varieties of sorghum, viz. Feterita 1931 (hereafter abbreviated to Feterita) at Wad Medani, and Debekri at Zeidab. At each site there were two factorial experiments.

Experiments 1 (Medani) and 2 (Zeidab)—tested the effects of 5 fertilizer, 5 weed-killer spray and 3 trap-crop treatments (total of $5 \times 5 \times 3 = 75$ plots).

(i) No fertilizers *v.* fertilizers, a mixture of 80 lb. N and 40 lb. P_2O_5/acre given at sowing, 3, 6 or 9 weeks later.

(ii) No weed-killer spray *v.* 0·8 and 1·6 lb. 2:4-D/acre sprayed either 2 or 3 weeks after sowing.

(iii) No trap crop *v.* Sudan grass grown for either 3 or 5 weeks before planting sorghum.

Experiments 3 (Medani) and 4 (Zeidab) tested the same factors as in Exps. 1 and 2 except the trap crops (total of $5 \times 5 = 25$ plots).

Experiments done in 1958

Experiment 5, at Wad Medani only, tested the effects of four levels of N and two of P (total of $4 \times 2 = 8 \times 5$ replicates $= 40$ plots). The sorghum varieties Dwarf White Milo and Wad Fahl were sown.

(i) Four levels of N = 0·0, 40·0, 80·0 and 120·0 lb. N/acre using urea (46·6% N).

(ii) Two levels of P = 0·0 and 40·0 lb. P_2O_5/acre.

Experiment 6, at Wad Medani only, tested the effects of five levels of N, two levels of P and two weed-killer treatments (total $= 5 \times 2 \times 2 = 20 \times 6$ replicates $= 120$ plots). Each plot was split—one-half was sown with Feterita and the other with Debekri.

(i) Five levels of N = 0·0, 20·0, 40·0, 60·0 and 80·0 lb. N/acre.

(ii) Two levels of P = 0·0 and 40·0 lb. P_2O_5/acre.

(iii) No weed-killer spray *v.* 0·8 lb. 2:4-D/acre sprayed 24 days after sowing. Spraying was delayed because of severe flooding after heavy rainfall.

Experiment 7, at Zeidab only, tested the effects of two levels of N, of P and of K and three weed-killer treatments (total $= 2 \times 2 \times 2 \times 3 = 24 \times 6$ replicates

= 144 plots). Each plot was split and sown with Feterita and Debekri. The amounts of N, P and K fertilizers were equivalent to 40 lb./acre of N, P_2O_5 and K_2O, respectively. One-third of the experiment was unsprayed, the rest was sprayed with 1·2 lb. 2:4-D/acre either 14 or 21 days after sowing.

Notes on experimental materials

(1) Sorghum varieties. Four were grown; according to Snowden's classification (Bacon, 1948) Feterita 1931 belongs to *Sorghum vulgare* Pers. var *caudatum* Stapf, Debekri and Wad Fahl to *S. vulgare* Pers. var. *durra* Stapf, and Dwarf White Milo to *S. vulgare* Pers. var. *subglabrescens* Schweinf. *et* Aschers.

(2) *Weed-killer sprays.* 'Fernoxone', a preparation containing 80% of the sodium salt of 2,4-dichlorophenoxyacetic acid plus a dispersing agent, was used, mixed with water and sprayed at a rate of 80 gal./acre from a knapsack sprayer. In the text the quantities of weed-killer refer to the active material which, for brevity, is called 2:4-D.

(3) *Fertilizers.* Sulphate of ammonia (21% N), single (21% P_2O_5) and triple (45% P_2O_5) superphosphate and sulphate of potash (49·5% K_2O) were usually used. They were placed in holes made between the rows of plants and then covered.

(4) *Trap crops.* Sudan grass which stimulates the germination of *S. hermonthica* and is subsequently parasitized (McGrath *et al.* 1957), was grown for varying periods before the land was cropped with sorghum.

Seeds were broadcast on to the flat at a rate of 40 lb./acre, the land was raked and then irrigated every 10 days. Three or five weeks later the grass was cut at ground-level—the foliage was removed but the roots were left *in situ.* After being dry fallowed for a few days, the land was planted with sorghum. The Sudan grass did not regenerate.

Growth observations

(*a*) *Sorghum.* The numbers of holes, made at sowing, with sorghum were counted frequently and from them and the harvest data the treatment effects were assessed. At harvest (Table 1) the two outside rows/plot were discarded. The heads of grain in the central row of each split plot (seven rows *in toto*) and of the two central rows of undivided plots (fourteen rows) were counted and weighed with about $\frac{1}{2}$ in. of peduncle. The straw was then cut at ground-level, weighed fresh and air-dried to constant weight. Only the fresh weights of grain and straw were taken from the rest of the plots. In the text, the yields refer to the weights of fresh unthreshed grain and air-dried straw.*

(*b*) *S. hermonthica.* Its incidence was assessed in two ways. First, the numbers of sorghum holes with aerial *S. hermonthica* were counted and expressed as percentages of the total number of holes per plot = % infections. They were not modified or 'weighted' to allow for varying numbers of parasitic stems/hole.

* The Sudanese units of measure are:
(i) of weight, the rotl = 0·99 lb. = 0·449 kg., 336 rotls of sorghum = 1 ardeb;
(ii) of area, the feddan = 1·038 acre = 0·420 ha.
For convenience the local measures have been converted to lb./acre taking the conversion factor as unity. 1 lb./acre = 0·18 kg./ha.

Secondly, *S. hermonthica*, both living and dead, was collected about 3 weeks before sorghum harvest and air-dried to constant weight.

Analysis of data

Many of the treatment effects are very large and their significances are obvious without being supported by detailed statistical analyses.

Often errors increased with the size of the means and the weights of both *S. hermonthica* and sorghum were then transformed for analysis to log $(n+1)$, where 'n' is the actual weight in g./plot or lb./acre. The text, however, usually omits the transformed data and the significant effects are marked by asterisks.

The percentage infections were usually analysed without being transformed, but were sometimes converted to angles.

Incidence of *Striga hermonthica**

Germination of *S. hermonthica* is stimulated by sorghum root exudates. In the present series of experiments sorghum seedlings usually appeared about 6 days after sowing and *S. hermonthica* 2–3 weeks later (Fig. 1). The infestations varied

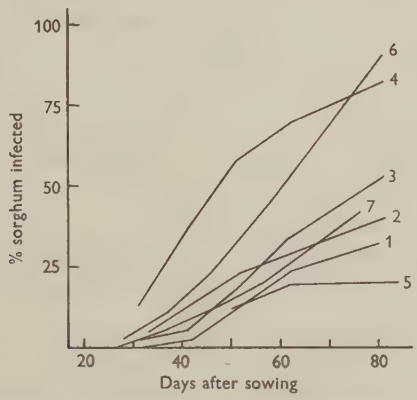

Fig. 1. Variations in the incidence of *S. hermonthica* in seven experiments done in 1957 and 1958.

considerably—they were particularly severe in Exps. 4 and 6, where the mean levels of infection reached at least 50% within 60 days of planting. Sometimes the percentage infections were affected by treatments but not the weights of parasite and vice versa.

(1) *Variety*. In 1958 the two varieties, Feterita and Debekri, were compared at both Medani and Zeidab. At Zeidab, where the infestation developed slowly, fewer Feterita plants were infected than Debekri (Table 2, Exp. 7) and this

* In the text three plants are frequently mentioned—*Sorghum vulgare* Pers., *S. sudanense* (Piper) Stapf. and *Striga hermonthica* (Del.) Benth. To avoid confusion, when abbreviated, they will be referred to as sorghum, Sudan grass and *S. hermonthica*.

difference was reflected by similarly significant differences in the weights of *S. hermonthica*, viz. 162 and 229 lb./acre on Feterita and Debekri, respectively.

At Medani (Exp. 6) the comparison was made on a site where the infestation built-up more rapidly and reached a higher mean level: 90% infected at Medani after 80 days against only 42% at Zeidab after 74 days. Although equal numbers of both varieties were infected in these conditions, *S. hermonthica* still developed more profusely on Debekri than Feterita, yielding 716 against 532 (±20·2) lb. of dry foliage/acre, respectively.

TABLE 2. *Incidence of* Striga hermonthica *on two lightly infested sorghum varieties at Zeidab, 1958 (Exp. 7)*

	% infection (days after planting)		
Variety of sorghum	37 days	56 days	76 days
Feterita	4·3	15·9	38·2 (*36·9*)*
Debekri	8·0	23·2	45·9 (*42·9*)

* Angular transformations, S.E. ±0·77

TABLE 3. *Effects of weed-killer sprays on the harvest weights of* Striga hermonthica *(lb./acre) in six experiments done in 1957–8*

		(*a*) Concentration of 2:4-D/acre		(*b*) Sprayed, weeks after sowing	
	No spray	0·8 lb.	1·6 lb.	2 weeks	3 weeks
Exp. 1	12·0	3·7*	1·2*	3·4*	1·6*
2	122·0	68·0	58·0	52·0	75·0
3	32·4	15·5	7·2*	19·0	3·7*
4	1060·0	1090·0	510·0*	940·0	660·0
7	390·0	—	—	110·0*	166·0*

Exp. 6—No spray: 900·0; 0·8 lb. 2:4-D/acre sprayed 24 days after sowing: 380·0*

* Differs significantly ($P = 0·05$) from unsprayed controls.

(2) *Weed-killer sprays.* 2:4-D sprays affected the mean development of *S. hermonthica* in crops of Feterita and Debekri equally (Exps. 6, 7). They always retarded the rate of new infections and usually decreased the eventual weights of *S. hermonthica*, although not always significantly. Sprays given either 2 or 3 weeks after planting, and before *S. hermonthica* appeared, were compared in five experiments. The data for 1957 given in Fig. 2 are the means of Exps. 1 and 3 at Medani, and of 2 and 4 at Zeidab. The data for Zeidab 1958 are from Exp. 7.

At Medani, spraying 3 weeks after planting retarded the development (Fig. 2) and decreased the weights of *S. hermonthica* significantly more than spraying after 2 weeks (Table 3). In Exp. 1 the transformed weights of *S. hermonthica* decreased from 2·43 ±0·21 in unsprayed crops to 1·84 and 1·37 (both ±0·15) in crops sprayed 2 and 3 weeks after sowing, respectively. The corresponding data for Exp. 3 are 2·86 ±0·28, and 2·58 and 1·91 (both ±0·20).

In both seasons at Zeidab, sprays given 2 and 3 weeks after sowing were equally effective. They significantly delayed the appearance of *S. hermonthica*, but the

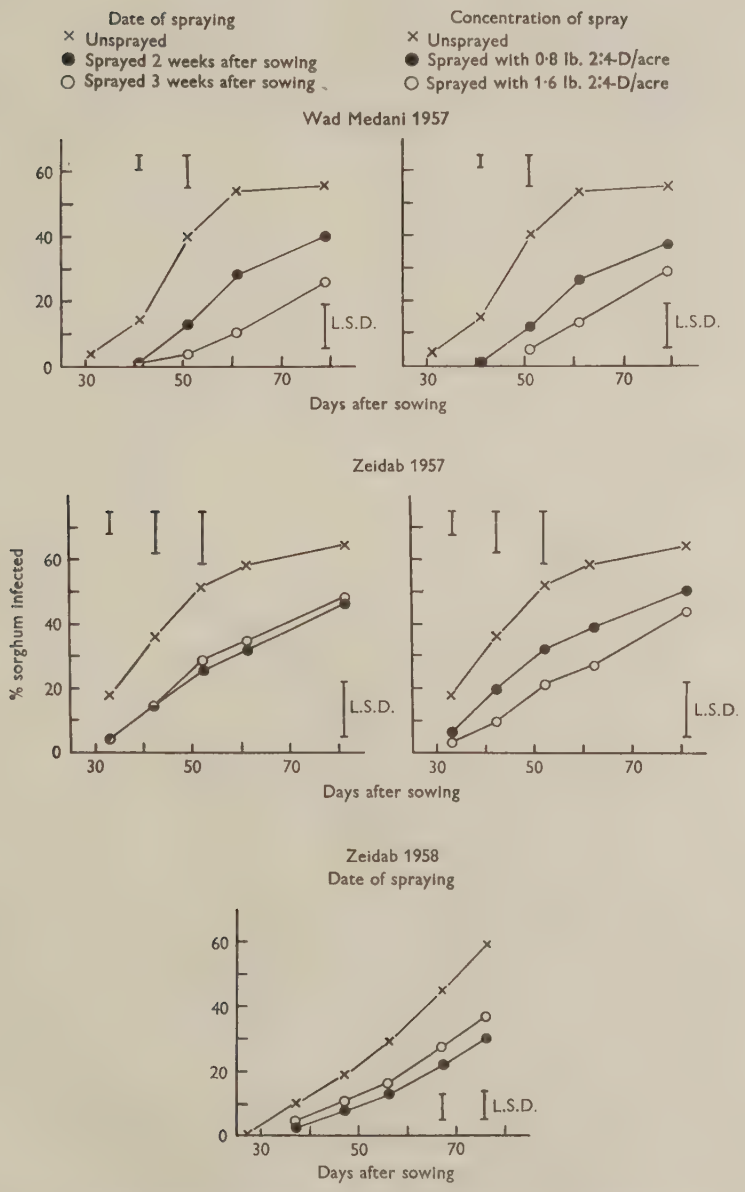

Fig. 2. Effects of 2:4-D sprayed on different occasions and at different rates on the numbers of sorghum plants infected with *S. hermonthica*.

smaller weights of the parasite in 1957 did not differ significantly from those in the unsprayed controls.

The higher concentration of 2:4-D, viz. 1·6 lb./acre, consistently retarded the rate of new infections more than the lower concentration of 0·8 lb., at both Medani and Zeidab. Similarly, in three of the four comparisons, twice significantly, weights of *S. hermonthica* were decreased more by the higher than the lower concentration (Table 3).

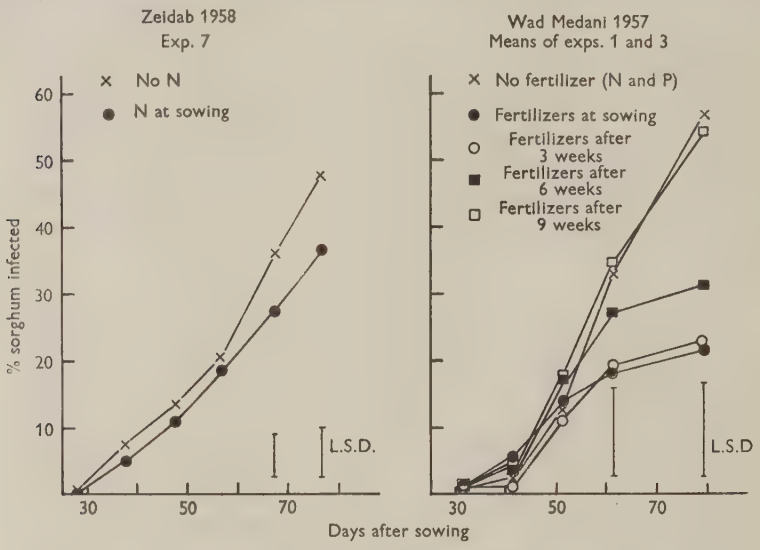

Fig. 3. Effect of fertilizers on the numbers of sorghum plants infected with *S. hermonthica*.

(3) *Fertilizers.* In the initial series of experiments (Exps. 1–4), done in 1957, a mixture of N and P was used because sorghum crops in different parts of the Sudan are known to respond to both. Fertilizers (N and P) given to Feterita at Medani delayed the appearance of new infections from the sixtieth day after sowing onwards (Fig. 3) and decreased the weights of *S. hermonthica*—early applications were more effective than late applications (Table 4). At Zeidab, however, applying mixed fertilizers to Debekri did not affect the percentage infections and when given 6 and 9 weeks after planting increased the weights of *S. hermonthica*, but never significantly. Because these contrasting results might reflect site effects and differing varietal reactions to fertilizers, experiments in 1958 were designed to test each of these possibilities. In addition, the responses to N, P and K were measured separately.

In Exps. 5, 6 and 7, done in 1958, neither phosphorus nor potash affected the incidence of *S. hermonthica* or sorghum yields. Therefore the effects of the mixed fertilizers used in 1957 can reasonably be attributed solely to N.

Although weed-killer sprays affected *S. hermonthica* equally on Feterita and Debekri, N had very different effects on different varieties. In Exp. 7 at Zeidab

the infestation of *S. hermonthica* developed slowly (Fig. 1), and without N similar numbers of Feterita and Debekri were infected. With N, however, the mean level of infection after the sixtieth day was again lower (Fig. 3) and significantly fewer Feterita plants were infected than Debekri. These effects are reflected by similarly different weights of *S. hermonthica* (Table 5). Thus in three experiments, Exps. 1,

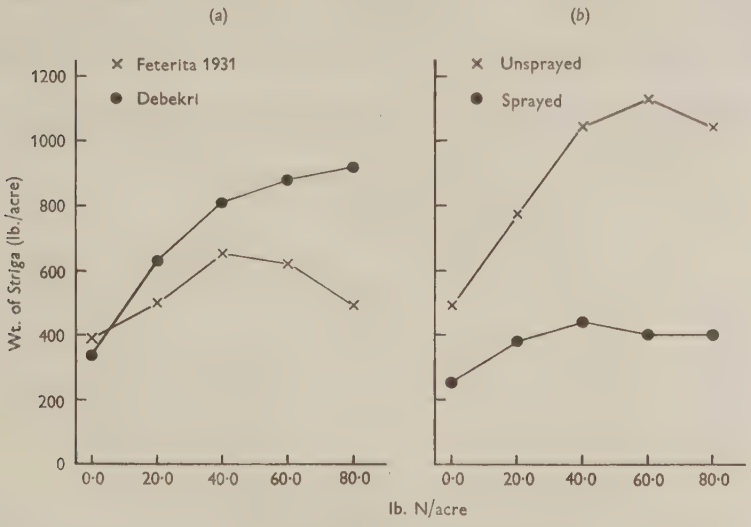

Fig. 4. Effect of N on the weights of *S. hermonthica* grown on (*a*) different sorghum varieties and (*b*) sprayed (2:4-D) and unsprayed crops (Exp. 6).

TABLE 4. *Effects of applying fertilizers* (N *and* P) *at different intervals after sowing on the weights* (*lb./acre*) *of* Striga hermonthica

(Transformed data in italics.)

	Site	Variety		No N	Fertilizers given (weeks after sowing) 0	3	6	9
Exp. 1	Medani	Feterita		16·1	0·6	0·7	1·2	12·8
			±0·21	*2·55*	*1·16*	*1·24*	*1·46*	*2·45*
3	Medani	Feterita		66·0	1·1	5·0	16·3	20·2
			±0·28	*3·16*	*1·40*	*2·06*	*2·56*	*2·66*
2	Zeidab	Debekri		77·0	52·0	27·0	166·0	129·0
			±0·29	*3·24*	*3·07*	*2·79*	*3·57*	*3·46*
4	Zeidab	Debekri		600·0	574·0	640·0	1390·0	930·0
			±0·16	*4·13*	*4·11*	*4·15*	*4·49*	*4·32*

3 and 7, done at different sites in different years and in which the mean rates of appearance of new infections, but not the weights of *S. hermonthica*, were similar, nitrogen consistently decreased the incidence of the parasite on Feterita. The different responses given to the same fertilizers in 1957 therefore probably reflect varietal and not site effects.

In Exp. 5 where the infestation again developed comparatively slowly, urea decreased the incidence of *S. hermonthica* on Dwarf White Milo and Wad Fahl in the same way as sulphate of ammonia on Feterita (Table 6).

Although fertilizers given to Debekri in Exps. 2 and 4 sometimes doubled the weights of *S. hermonthica*, the increases are not significant. However, on very infertile soil, where the infestation of *S. hermonthica* developed early and severely (Exp. 6), N significantly increased the weights of parasite on both Feterita and Debekri (Fig. 4). This result contrasts with the effect of N on less severely para-

Table 5. *Effect of* N *together with* (1) *varieties*, (2) P, *and* (3) *weed-killer sprays on the incidence of* Striga hermonthica *on comparatively fertile soil* (Exp. 7).

(Weights of *S. hermonthica*, '*n*' lb./acre, transformed to log (*n*+1): percentage infections transformed to angles.)

(1) N and variety

	% infection			Wt. of *S. hermonthica*		
	Feterita	Debekri	Mean (±1·91)	Feterita	Debekri	Mean (±0·057)
N −	42·4	45·6	44·0	2·37	2·43	2·40
N +	31·5	40·2	35·8	2·05	2·29	2·17
Mean (±0·77)	36·9	42·9	— (±0·024)	2·21	2·36	—
S.E. for: Horizontal comparisons	±1·10				±0·034	
Vertical and diagonal	±2·06				±0·061	

(2) N and P

	% infection (±2·69) P			Wt. of *S. hermonthica* (±0·080) P		
	—	+	Mean (±1·91)	—	+	Mean (±0·057)
N −	47·5	40·5	44·0	2·47	2·33	2·40
N +	36·0	35·7	35·8	2·17	2·17	2·17
Mean (±1·91)	41·7	38·1	— (±0·057)	2·32	2·25	—

(3) N and weed-killer sprays

% infection after 76 days (±3·30)

		Sprayed, with 1·2 lb. 2:4-D/acre (days after sowing)		
	No spray	14 days	21 days	Mean (±1·91)
N − (1)	60·6	34·9	36·6	44·0
N + (2)	44·3	26·9	36·3	35·8
Mean (±2·33)	52·4	30·9	36·4	—
Diff. (2)−(1)	16·3	8·0	0·3	—

sitized crops of Feterita, but the increases on Feterita were smaller than on Debekri. Fig. 4 shows that the usual decreases expected after applying N to Feterita occurred when more than 40 lb. N were given per acre. The mean weights of *S. hermonthica* parasitizing both sprayed and unsprayed Feterita and Debekri increased with additions of up to 40 lb. N/acre; further additions checked the development of *S. hermonthica* on Feterita, whereas on Debekri it continued to develop with up to

80 lb./acre (Fig. 4). Clearly the effect of N on the growth of *S. hermonthica* differs with the host variety and possibly with the level of soil fertility. Adding up to 40 lb. N/acre to Feterita, which yielded only 109 lb. grain/acre when unmanured, maintained a greater bulk of 'susceptible' plants and only after this level of fertility was reached did its resistance show so that the weight of parasite decreased. These differences occurred even though N did not affect the rate of new infections.

The effect of N differs with variety and is also modified by weed-killer sprays. On the severely affected crops in Exp. 6 *S. hermonthica* increased significantly less

TABLE 6. *Mean effects of different amounts of* N, *given in Exp. 5 as urea, on the incidence of* Striga hermonthica *in crops of Dwarf White Milo and Wad Fahl*

(Transformed data in italics.)

lb. N/acre ...	0·0	40·0	80·0	120·0
% infection after 84 days	37·6	30·7	8·5	3·6
Wt. of *S. hermonthica* (lb./acre) after 91 days	18·7 (±0·22) *(2·32)*	8·6 *(1·98)*	2·5 *(1·45)*	0·9 *(1·06)*

TABLE 7. *Effects of weed-killer sprays and nitrogen fertilizers on the weights of* Striga hermonthica *(lb./acre) developed on two sorghum varieties in very infertile soil at Wad Medani,* 1958 (Exp. 6)

		lb. N/acre							
		0·0	20·0	40·0	60·0	80·0	Mean	(±20·2)	(±32·4)
Feterita	Unsprayed	540	685	1000	965	800	798	Feterita	Unsprayed
	Sprayed	245	315	305	285	180	266	532	879
Debekri	Unsprayed	420	825	1055	1260	1240	960	Debekri	Sprayed
	Sprayed	255	430	565	505	605	472	716	369
	Mean (±51·0)	365	564	731	754	706	—		

when N was given to sprayed than unsprayed crops (Fig. 4). Adding 20, 40 and 60 lb. N/acre to unsprayed crops successively increased the mean weights of parasite from 480 to 755, 1028 and 1113 lb./acre, respectively. On sprayed crops the amount of *S. hermonthica* was not increased significantly further by adding more than 20 lb. N which increased the infestation from 250 to 373 lb./acre. The contrast between Feterita and Debekri is greater when the weights of *S. hermonthica* on sprayed plots alone, and not the means of sprayed and unsprayed, are compared (Table 7). The infestations on sprayed Feterita were never significantly increased by N and with 80 lb./acre were smaller than on plots without N. On sprayed Debekri the amount of *S. hermonthica* continued to increase with increasing amounts of N but less rapidly than in the unsprayed crop.

Where *S. hermonthica* developed late in the season, as in Exp. 7, N decreased its incidence more on unsprayed than sprayed crops. The levels of infection fell by 16·3, 8·0 and 0·3% on unsprayed crops and crops sprayed 2 and 3 weeks after

sowing, respectively (Table 5). These effects were not reflected by comparable differences in the weights of *S. hermonthica*.

(4) *Trap crops.* Roots of Sudan grass grown for 5 weeks, but not for 3, were infested with numerous seedlings of *S. hermonthica* at both Zeidab and Medani. Only a small percentage of the parasites came above ground.

As was to be expected, the incidence of *S. hermonthica* in succeeding sorghum crops was significantly affected only by 5 weeks' trap cropping. The percentage infections decreased from 51·2 in the untreated to 22·7% at Zeidab and from 46·4 to 16·1% at Medani (Fig. 5). These differences are reflected in comparable decreases in the weights of *S. hermonthica* (Table 8).

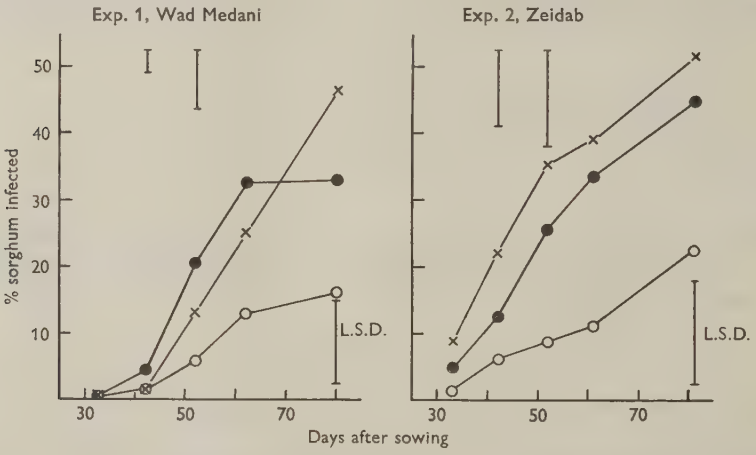

Fig. 5. Effect of trap cropping with Sudan grass on the percentages of succeeding sorghum crops infected with *S. hermonthica*. ×—×, sorghum grown without trap cropping; ●—● and ○—○, sorghum following 3 and 5 weeks' trap cropping, respectively.

TABLE 8. *Effect of previous trap crops of Sudan grass on the weights of* Striga hermonthica *(lb./acre) in succeeding sorghum crops*

(Transformed weights in italics.)

	No Sudan grass	Sudan grass grown for 3 weeks	Sudan grass grown for 5 weeks
Exp. 1 (±*0·16*)	6·2 (*2·14*)	3·3 (*1·88*)	0·8 (*1·30*)
Exp. 2 (±*0·22*)	198·0 (*3·64*)	115·0 (*3·41*)	18·5 (*2·62*)

Sorghum yields

(1) *Variety.* The varieties Feterita and Debekri behaved differently at Zeidab and Medani. In Exp. 6, done at Medani, Feterita gave significantly larger mean yields than Debekri—881 and 3215 lb. of grain and straw per acre against 534 and 2150 lb. from Debekri. In contrast, Debekri at Zeidab yielded significantly more

straw than Feterita, 5750 *v.* 5060 lb./acre, but their grain yields were similar, viz. 2380 lb./acre (Exp. 7).

(2) *Weed-killer sprays.* 2:4-D sprays usually temporarily check sorghum growth but the harm done is usually offset by the benefit gained by the greater check to developing *S. hermonthica* (Wilson-Jones, 1953*a*). For example, spraying increased average grain yields by 530% from 225 to 1208 lb./acre in Exp. 4 (Table 9) and by 400% from 296 to 1120 lb./acre in Exp. 6 (Table 10). The absolute increases of straw were greater than of grain, but the relative increases were smaller.

In Exp. 1 at Medani spraying retarded the development of new infections (Fig. 2) and decreased the weight of *S. hermonthica* (Table 3). Sorghum yields, however, were not significantly affected, probably because the small infestations on the untreated controls, viz. 12·0 lb. *S. hermonthica* per acre, were not damaging. The higher concentration of 2:4-D, 1·6 *v.* 0·8 lb./acre, sprayed 2 and 3 weeks after sowing, may have caused more damage than usual by interacting with flooding caused by a rainstorm a few days after the later spraying. Many plants died and the mean stands decreased from about 450 holes with sorghum in unsprayed plots and plots sprayed with the lower concentration, to 405 holes in plots sprayed with the higher concentration of 2:4-D.

In the other experiments the effects of 2:4-D on sorghum yields seem to be related to the date of appearance of *S. hermonthica* rather than to its final weight. Where the mean level of infection reached 20% within 50 days of sowing, as in Exps. 2, 4 and 6 (Figs. 1, 2) weed-killer sprays increased both grain and straw yields (Table 9). Where the infestations developed slowly, weed-killer sprays increased, and *S. hermonthica* presumably decreased, only grain yields. The infestation of *S. hermonthica* on unsprayed Debekri reached 440 lb./acre in Exp. 7, but it developed later and was less damaging than 122 lb. of *S. hermonthica* in Exp. 2 (Figs. 1, 2 and Table 9). In Exp. 7 spraying increased the average grain yield of Debekri by 500 lb. and had no effect on weights of straw. In Exp. 2 it increased grain and straw yields by about 800 and 950 lb., respectively. These effects emphasize the greater damage done by early than by late infections.

Although the effects varied considerably from one experiment to another, the means given in Table 9 show that sprays given either 2 or 3 weeks after sowing and using either 0·8 or 1·6 lb. 2:4-D/acre were equally effective.

In Exp. 6, but not Exp. 7, the effect of spraying on sorghum yields differed with variety even though the infestations were equally affected. Spraying increased the grain and straw yields of Feterita by 936 and 2490 lb./acre, respectively, and of Debekri by only 711 and 1960 (Table 10).

(3) *Fertilizers.* P and K when tested separately in Exps. 5, 6 and 7 did not affect yields. The responses to the mixed fertilizers (N and P) in Exps. 1–4 were therefore probably caused by N.

As infestations increase in severity there is a tendency (*a*) for the grain and straw yields without N to decrease and (*b*) for the relative, but not absolute, yield responses to N to increase progressively. In Exps. 4 and 6 where the mean levels of infection reached 50% within 60 days of planting (Fig. 1), the plots without N yielded 265

Table 9. *Mean effects of weed-killer sprays on sorghum yields (lb./acre)*

	Site	Variety	A. Grain: No spray	A. Grain: (a) Concentration of 2:4-D/acre: 0·8 lb.	A. Grain: (a) Concentration of 2:4-D/acre: 1·6 lb.	A. Grain: (b) Sprayed, weeks after sowing: 2 weeks	A. Grain: (b) Sprayed, weeks after sowing: 3 weeks	B. Air-dry straw: No spray	B. Air-dry straw: (a) Concentration of 2:4-D/acre: 0·8 lb.	B. Air-dry straw: (a) Concentration of 2:4-D/acre: 1·6 lb.	B. Air-dry straw: (b) Sprayed, weeks after sowing: 2 weeks	B. Air-dry straw: (b) Sprayed, weeks after sowing: 3 weeks
Exp. 1	Medani	Feterita	2400	2560	2410	2420	2550	5400	5440	5030	5290	5180
2	Zeidab	Debekri	1600	2480*	2330*	2420*	2380*	4650	6020*	5150	6010*	5160
3	Medani	Feterita	1290	2240*	1900*	1950*	2200*	4630	4810	4590	4630	4770
4	Zeidab	Debekri	225	675	1740*	1180*	1230*	2500	3900*	5850*	4880*	4870*
Overall means of spray concentrations (Exps. 1–4)			**1380**	**1990**	**2095**	—	—	**4300**	**5040**	**5160**	—	—
7	Zeidab	Feterita and Debekri	2030	—	—	2550*	2490*	5440	—	—	5380	5400
Overall means of dates of spraying (Expts. 1–4, 7)			**1510**	—	—	**2105**	**2170**	**4520**	—	—	**5240**	**5080**

* Differs significantly ($P = 0{\cdot}05$) from unsprayed controls.

and 100 lb. of grain per acre (Table 11). They yielded 840 lb. in Exp. 3 where the infection mean reached 50% after 80 days, whereas in the remaining experiments, where the 50% level was never reached, the lowest yield was 905 lb. of grain per acre.

80 lb. N/acre given at sowing increased grain yields of the severely infested crops in Exps. 4 and 6 by 1325 lb. from an average of 180 to 1505 lb./acre—an increase of ×8·2. They increased the yields of the less severely infested crops in Exps. 1,

TABLE 10. *Effect of* 2:4-D *sprays on the yields of two sorghum varieties at Wad Medani,* 1958 (*Exp.* 6)

(1) Grain (lb./acre)

	Unsprayed	Sprayed	Mean (±29·9)
Feterita	413	1350	881
Debekri	179	890	535
Mean (±36·2)	296	1120	—

(2) Air-dry straw (lb./acre)

	Unsprayed	Sprayed	Mean (±63·0)
Feterita	1970	4460	3215
Debekri	1170	3130	2150
Mean (±97·5)	1570	3795	—

TABLE 11. *Mean effects of fertilizers given at sowing on sorghum yields* (*lb./acre*)

	A. Grain					B. Air-dry straw				
		Wt. of N/acre					Wt. of N/acre			
	No N	20 lb.	40 lb.	60 lb.	80 lb.	No N	20 lb.	40 lb.	60 lb.	80 lb.
Exp. 1	1140	—	—	—	3530*	3270	—	—	—	6590*
2	905	—	—	—	3510*	3350	—	—	—	7010*
3	840	—	—	—	3020*	2540	—	—	—	6040*
4	265	—	—	—	1630*	2940	—	—	—	5580*
6	100	305*	645*	1110*	1380*	751	1990*	3080*	3830*	3770*
7	1810	—	2910*	—	—	4800	—	6010*	—	—

* Differs significantly ($P = 0·05$) from unmanured controls.

2 and 3 by 2390 lb. from an average of 960 to 3350 lb./acre—an increase of ×3·5. Even in Exp. 1 where the infestation was too small to cause losses (judging by the results of 2:4-D sprays) N increased grain yields by 300% from 1140 to 3530 lb./acre.

Regardless of the severities of the infections, fertilizers given at sowing or after 3 weeks in Exps. 1–4 always increased yields of grain and straw more than fertilizers given later (Fig. 6).

Where sorghum was infected early and severely and where the treatments were highly replicated, the responses to N were significantly modified by both variety and spray treatments (Exp. 6). N applied to the less severely infected sprayed crops gave larger yield increases than when applied to unsprayed crops. 80 lb. N/acre increased the grain and straw yields of sprayed crops from 164 and 1170 lb. to 2170 and 5450 lb./acre, respectively, and of unsprayed crops from 38 and 350 lb. to only 590 and 2100 lb./acre. Similarly N increased the yield of Feterita, the less susceptible variety, more than of Debekri (Fig. 7). Further, whereas *S. hermonthica*

continued to increase on Debekri with each addition of N, from 20 to 80 lb./acre (Fig. 4), the grain and straw yields were not significantly increased by more than 60 lb. N (Fig. 7). In contrast, the mean weights of *S. hermonthica* on Feterita decreased after adding 40 lb. N but the yields of sorghum continued to increase.

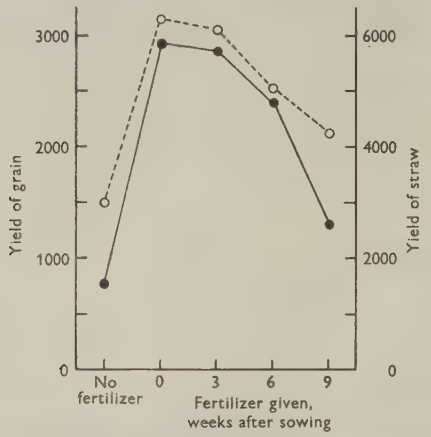

Fig. 6. Effect of mixed fertilizers (N and P), given at different intervals after sowing, on sorghum yields, lb./acre (Exps. 1–4). ● = grain; ○ = straw.

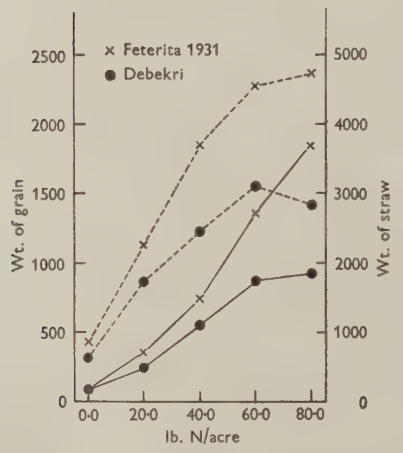

Fig. 7*a*

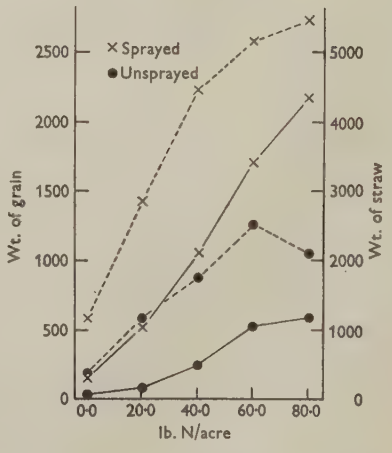

Fig. 7*b*

Fig. 7. Effect of N on the yields of (*a*) different sorghum varieties and (*b*) sprayed (2:4-D) and unsprayed crops (Exp. 6). ——, grain; - - -, straw.

In the other experiment comparing varieties, Exp. 7, *S. hermonthica* developed comparatively slowly. N increased the grain yields of Feterita and Debekri similarly, but the larger increases of Feterita straw were again associated with the bigger decrease in the amount of parasite. Thus N increased straw yields and

decreased weights of *S. hermonthica* by 1450 and 122 lb./acre, respectively, when given to Feterita and by 960 and 75 lb./acre when given to Debekri. Similarly, the effect of N was larger on unsprayed crops, where it greatly decreased the incidence of *S. hermonthica*, than on sprayed crops, where its effect on disease was less. Decreases of 16·3, 8·0 and 0·3% in the levels of infection are associated with increases of 1660, 1250 and 750 lb. of straw per acre.

(4) *Trap crops.* Sudan grass grown for 5 weeks greatly decreased the incidence of *S. hermonthica* in succeeding sorghum crops (Fig. 5), and where the control plots were heavily infected the grain yields were correspondingly increased. In Exp. 2 the grain yields increased systematically, but not significantly, from 1940 (*1·64*) in the untreated to 2270 (*1·71*) and 2510 (1·75 ± 0·047) lb./acre after 3 and 5 weeks trap cropping, respectively. Sorghum grown on unmanured plots and following 5 weeks trap cropping showed signs of nitrogen deficiency within 3 weeks of planting. On these infested and infertile soils the benefit gained by decreasing the

TABLE 12. *Effects of* N *on straw yields of crops on which* Striga hermonthica *developed late* (*Exp.* 7)

(1) N × weed-killer sprays

Straw lb./acre (± 194)

		Sprayed, weeks after sowing		
	No spray	2 weeks	3 weeks	Mean (± 112)
N − (1)	4610	4750	5050	4800
N + (2)	6270	6000	5750	6010
Mean (± 137)	5440	5380	5400	—
Diff., (2)−(1)	1660	1250	700	—

(2) N × sorghum variety

	Feterita	Debekri	Mean (± 112)
N − (1)	4340	5270	4800
N + (2)	5790	6230	6010
Mean (± 62)	5060	5750	—
Diff., (2)−(1)	1450	960	—

S.E. for: Horizontal comparisons, ± 88
Vertical and diagonal, ± 128

incidence of *S. hermonthica* by trap cropping is masked by the harm done by removing nutrients.

Addendum: components of grain yield. Judging by the increases already described, the major problem on land infested with *S. hermonthica* is to increase the weight of grain—quality might possibly be considered later. To see how the increases were achieved, analyses were made of the numbers and weights of heads of grain. For brevity, only the salient features are described.

(1) Variety. Where the infestation of *S. hermonthica* was severe (Exp. 6) the differing yields of Feterita and Debekri (Table 10) parallel similar differences in

their numbers of heads per hole. Feterita and Debekri yielded *c.* 880 and 525 lb. of grain per acre and developed 2·1 and 1·3 heads per hole, respectively.

(2) Weed-killer sprays. The increased grain yields following the control of late (Fig. 1) and sparse infestations of *S. hermonthica* by 2:4-D are mainly attributable to larger heads—their average weights were increased by *c.* 150% (Exp. 3). In contrast, when severe infestations developed early both numbers and sizes of heads were affected. In Exp. 4 sprays given either 2 or 3 weeks after planting acted similarly but the increases caused by 1·6 lb. of weed-killer per acre were at least twice as large as those caused by 0·8 lb. The numbers of heads per hole increased from 1·6 in the unsprayed to 2·5 and 4·2 and the weights of 1000 heads from 15·8 to 29·9 and 44·8 lb. with successively more concentrated sprays. Sprays affected the two varieties differently—the numbers of Feterita heads were increased more, and their weights less, than heads of Debekri (Exp. 6).

(3) Fertilizers. Applying increasing doses of N increased the 1000 head weights of Feterita and Debekri equally from a mean of 11·5 without N to 19·3, 27·0, 39·1 and 45·3 lb. with 20, 40, 60 and 80 lb. N/acre, respectively (Exp. 6). The numbers of Feterita heads per hole, however, increased more than those of Debekri—from 0·9 and 0·6 in the absence of N to 3·1 and 1·6 with the highest dose. N had more effect on sprayed than unsprayed crops. The mean numbers of heads increased from 1·2 to 3·1 after adding 80 lb. N/acre to sprayed crops but from 0·4 to only 1·6 in unsprayed crops. Similarly the 1000 head weights of sprayed crops went from 13·3 to 61·5 and of unsprayed from 9·4 to only 28·8 lb.

Fertilizers given at sowing and 9 weeks later increased equally the number of heads in a lightly infested crop (Exp. 3). In Exp. 4, where the crop was more severely infected and yielded less without manure, fertilizer applied at sowing gave the largest increase—from 1·4 heads per hole without fertilizers to 5·7 and 1·8 with fertilizers given at sowing and 9 weeks later, respectively. In Exps. 3 and 4, the 1000 head weights were significantly increased by fertilizers given up to 6 weeks after sowing but not when given later.

Discussion

Much is known of the germination of *Striga* seeds and of its relation to exudates produced by a wide range of plants (Saunders, 1933; Brown *et al.* 1952; Vallance, 1951), also of the organic contact between the parasites' haustoria and the hosts' roots (Stephens, 1912*a*, *b*; Saunders, 1933; Uttaman, 1950), but little is known of the host–parasite relation after parasitism has been established.

In variety trials in Tanganyika, Doggett (1956, 1958) found that fewer aerial shoots of *S. hermonthica* and *S. asiatica* developed on the sorghum variety Dobbs than on other types. In my experiments Feterita was usually less severely infected than Debekri and the development of *S. hermonthica* on Feterita, Dwarf White Milo and Wad Fahl varied greatly with fertilizer treatments. On lightly infested soils of comparatively high fertility, nitrogenous fertilizer repeatedly decreased the development of aerial stems of *S. hermonthica*. Similarly, by increasing the concentrations

of a composite fertilizer, containing N, P and K, given to sorghum grown in sand cultures, Solomon (1952) found that the numbers of aerial shoots of *S. asiatica* and *S. densiflora* progressively decreased. These results might possibly be explained by straightforward competition in which the host is more successful when manured. However, this seems unlikely because instead of developing more profusely when crops were widely spaced, as most weeds would, Saunders (1933) and Crowther (1942) found that populations of *Striga* spp. decreased.

Even in sorghum crops that from the numbers of aerial stems seem to be heavily infected with *S. hermonthica*, only a small proportion (*c.* 5%) of the parasites established on the hosts' roots show above ground (Andrews, 1945). Unfortunately the total numbers of established root parasites were not counted either in my experiments or in Solomon's. Because Andrews found that the numbers of parasites are directly proportional to the size of the host's root system, the differing numbers of aerial stems on sorghum differently manured probably reflect changes in the ratios of emerged:unemerged parasites—the ratio progressively decreasing with increasing amounts of fertilizer.

In addition to affecting the ratios of emerged:unemerged seedlings, the host's nutrition affects the flowering of the parasite. Aerial stems of *S. asiatica* and *S. densiflora*, which developed on sorghum given high concentrations of fertilizers, rarely flowered (Solomon, 1952). I observed similar effects in Exps. 1 and 3 where *S. hermonthica* developed profusely on unmanured crops, but its growth was restricted when N was given at sowing and shortly afterwards—the stunted aerial shoots produced few flowers. This is also how the parasite looks when it attacks legumes—plants rich in N. Again, only about 2% of the parasites of *Dolichos lablab* L., *Arachis hypogea* L. and *Vigna unguiculata* Walp. emerged above ground; the shoots were no more than 1 cm. long and rarely flowered (Andrews, 1947).

When *S. senegalensis* was repeatedly hand-weeded from severely infected sorghum, new parasites continued to emerge giving a mean of 112,000 stems per acre; where it was left untouched only 26,000 appeared (Butler, 1957*b*). This observation suggests that parasites compete for some host material or materials which enable them to elongate, produce an aerial stem, and flower. Removing a parasite that has emerged above ground allows its supply of material to stimulate the development of other parasites. The numbers of *S. hermonthica* seedlings per host increase rapidly in the first 5 weeks after sowing and then remain constant (Andrews, 1945), suggesting that already-established parasites compete for nutrients.

The nature of the essential materials that parasites derive from their hosts is unknown. Presumably they can neither be synthesized by the parasite nor directly absorbed from soil by its vestigial roots, which are usually characterized by the absence of root hairs (Stephens, 1912*b*).

The effect of N probably varies with soil fertility. Poor growth of the host, even after giving fertilizers, probably means that the early stimulus of the host has simply lead to infection by more *Striga* than before. This possibly explains the results in Exp. 6, where N given to the variety Feterita unexpectedly increased the

development of *S. hermonthica*. An extreme example of competition was reported by Saunders (1933), who found that *S. asiatica* rarely appeared above ground when maize was very heavily infected.

In many of the areas in Africa where *Striga* spp. are important, sorghum is grown repeatedly without fertilizers. Thomas (1943), Andrews (1945) and Portères (1948, 1952) suggested that, in these areas, the impoverished soils are the primary cause of severe *Striga* infestations and of poor yields. On the other hand, Wilson-Jones (1953*b*) considers *Striga* to be the primary cause of yield losses. My experiments support the suggestion that damaging infestations of *Striga* are correlated with N deficiency. *Striga* was never important until sorghum gave at least a 300% grain yield response to 80 lb. N/acre. When the response to N was less than 300%, the effects of weed-killer sprays in retarding and decreasing the development of *S. hermonthica* infestations were not reflected in increased yields. However, when the response to N was greater than 300%, the weed-killers increased yields. This relation between inherent soil fertility and *Striga* infestations may possibly be of value in attempting to prevent *Striga* from increasing in land which at present is relatively free. Thus, if crops are manured, there is every possibility of obtaining substantial yield increases while checking the development of *Striga*. The consequences of applying N resemble those on the balance between cereals and the fungus parasite *Ophiobolus graminis* Sacc. As with *Striga*, the severity of take-all, caused by *O. graminis*, decreased with increasing amounts of N (Glynne, 1951) and the magnitude of the effect is again closely related to its date of application (Salt, 1959).

Although the discussion has centred upon the use of N, there is no reason to suppose that were P and K deficient they would not act similarly. The host–parasite relation is complex and doubtless depends upon other soil factors in addition to nutrients—for example, soil texture and water supply. Heavy clays, for example, are unfavourable for the development of *S. asiatica* (Nelson, 1958).

The use of weed-killers against *Striga* has been reviewed by Wilson-Jones (1953*a*) and Williams (1958). They usually decrease the final weight of *Striga*, but probably increase sorghum yields mainly by protecting the host in its early stages, which is also suggested by Tarr's (1957) experiments which showed that spraying ridges on which sorghum was planted was more effective than spraying furrows. Selective weed-killers—for example, 2:4-dichlorophenoxyacetic acid (2:4-D)—are particularly valuable because in addition to affecting *Striga* seedlings which have emerged they also kill those still below ground-level (Kumar, Solomon & Rao, 1949). After considering possible damage done by these materials to sorghum, Wilson-Jones (1954) recommended spraying 2–3 weeks after sowing with 1 lb./acre of the sodium salt of 2:4-D. In my experiments concentrations of spray varying from 0·8 to 1·6 lb./acre were tested when applied either 2 or 3 weeks after sowing, but without giving clear-cut differences between the rates and dates of application. All combinations of rate and date of spraying retarded and usually decreased *S. hermonthica* with corresponding sorghum yield increases.

The root exudates of plants in a wide range of families, both mono- and di-

cotyledonous (McGrath *et al.* 1957), stimulate *Striga* seeds to germinate. By their subsequent reactions these plants are divided into three groups, where (*a*) parasitism is not established, (*b*) *Striga* parasitizes the host but rarely flowers, and (*c*) *Striga* parasitizes the host and flowers profusely. Thus cotton, *Gossypium barbadense* L., stimulates the germination of *S. hermonthica* but is not parasitized; *Dolichos lablab* is parasitized by *S. hermonthica* which rarely flowers; sorghum (*vulgare*) is parasitized and *S. hermonthica* flowers profusely.

Plants which stimulate germination but do not allow parasites to flower appear particularly suitable as trap crops, but in practice it is the fully susceptible types that have been used most successfully. In Rhodesia, Timson (1931, 1938) found that Kaffir corn (*Sorghum vulgare*), Sudan grass (*Sorghum sudanense*) and Rhodesian Sudan grass (*Sorghum arundinaceum*) are effective trap crops for eliminating *Striga asiatica* from lands repeatedly planted with maize. The trap crops are destroyed about 6 weeks

TABLE 13. *Effects of* 80 *lb.* N/*acre given at sowing and* 2:4-D, *sprayed* 24 *days after sowing, on the yields of two sorghum varieties, Feterita* 1931 *and Debekri, severely affected by* Striga hermonthica (*Exp.* 6)

	Feterita 1931		Debekri	
	No N	80 lb. N	No N	80 lb. N
	Yield of grain (lb./acre)			
Unsprayed	52	875	23	300
Sprayed	165	2805	160	1535
	Yields of straw (lb./acre)			
Unsprayed	465	2750	210	1445
Sprayed	1260	6700	1070	4190

after planting—sufficient time for *Striga* to germinate but not to flower. In my experiments Sudan grass grown for 5 weeks decreased the incidence of *S. hermonthica* in succeeding sorghum crops, on both lightly and heavily infested soils, but without significantly affecting grain yields. The good done by Sudan grass in diminishing *S. hermonthica* was offset because it used the available nutrients. As Timson (1945) has already stressed, trap cropping to eliminate *Striga* should be coupled with a programme for improving soil fertility.

The relative yield effects of parasites with and without aerial stems are unknown. Solomon (1952) found that sorghum losses were directly related to the numbers of aerial stems of *S. asiatica* and *S. densiflora*. But, because seedlings of *S. hermonthica* without aerial stems decrease yields of legumes—for example, *Dolichos lablab* and *Arachis hypogea* (Andrews, 1947)—they also probably affect sorghum, although less than parasites with aerial stems.

On well-grown sorghum, *Striga* spp. have little effect (Solomon, 1952), but in severe infestations there is no doubt that they cause crop losses. Equally it is true that, even if they were eliminated, many of the worst-infested soils would support only a very poor crop. In Nigeria, sorghum yields were increased four times by hand-weeding *S. senegalensis*, but still only reached 125 lb. of grain per acre

(Butler, 1957*a*). On these lands fertilizers give appreciable relative yield increases but their effects on *Striga* are uncertain. However, when fertilizers and weed-control measures are used together, very large and spectacular increases of both grain and straw may be achieved. On severely infested soil at Medani (Exp. 6) weed-killer sprays and 80 lb. N/acre increased grain yields of Feterita from 52 to 165 and 875 lb./acre, respectively (Table 13). Together they gave 2805 lb./acre—an increase of × 54.

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THE EFFECTS OF THE SIZE, POSITION AND MATURATION PERIOD OF INFLORESCENCES AND FRUITS ON ABNORMAL PIGMENTATION IN THE TOMATO VARIETY POTENTATE

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(With 4 Text-figures)

A modification to an earlier classification of pigmentation abnormalities, enabling it to be used for varieties other than Potentate, is discussed. It is shown for Potentate that the proportion of fruits giving uniform ripening colour declines with increasing fruit weight, while the proportion of Grade C fruits steadily increases. The occurrence of Grade A fruits, however, is maximal in fruits of 80 to 120 g. The occurrence of pigmentation abnormalities was shown to be independent of the length of the maturation period of the fruit. It was found that Grade A fruits occurred with greater frequency in fruits developing on upper inflorescences, while Grade C fruits occurred more frequently on lower inflorescences. The number of fruits in an inflorescence was shown to have no consistent relation to pigmentation abnormalities, nor was there any relation between pigmentation and the position of fruits within the inflorescence. Frequent examples of an antithetic relation between the occurrence of Grades A and C symptoms are noted, suggesting that conditions that are favourable for the development of the one are unfavourable for the development of the other.

In a previous paper the types of pigmentation abnormalities occurring in fruits of the tomato variety Potentate were classified and a fruit-grading scheme suitable for large-scale experimental work was outlined (Cooper, 1958).

As a result of further experience of this grading scheme it has been found possible to extend its use to varieties other than Potentate if a minor modification is made. Some varieties tend to bear a large number of fruits that are uniformly red except for small fragmented green areas of irregular shape of the kind shown by fruit 2 in the plate illustrating the paper referred to above. This greenness is confined to the surface layers of the fruit. It disappears very quickly, and by the time the fruit is fully mature it is uniformly red. Consequently, for varieties where large numbers of fruits show this abnormality, it has been found advisable to include this very transient condition not in Grade A, as originally proposed for the variety Potentate (in which it occurs only rarely), but in Grade UC. If this is not done when fruits from other varieties are being graded the error introduced into Grade A is considerable.

An error is also introduced into Grade A by the inclusion in that grade of misplaced Grade C symptoms, as occasionally a green area with the symptoms of Grade C occurs near the calyx. Typical Grade A fruits have the calyx completely surrounded by a ring of dark-green tissue, and the error introduced into this grade

by the inclusion of misplaced Grade C symptoms can be largely eliminated if only those fruits are included in Grade A that have a green area extending around the calyx for more than half the circumference.

In the data presented in this paper the above modifications were not made as they were developed during the course of the work.

For 3 years detailed observations were made on the fruits of Potentate plants grown under approximately commercial conditions. The weight (to the nearest gramme) and the colour grade of each individual fruit were recorded, and a note made of the truss from which it was picked and the position of the fruit within the truss.

In Fig. 1 (top) the number of uniformly coloured fruits of each weight (at 1 g. intervals up to 200 g.) has been shown expressed as a percentage of the total number of fruits picked of each weight. Similar curves are also shown for the numbers of Grades A and C fruits picked (Fig. 1, bottom and middle). The values from the year in which the size relation for each grade is least-well defined have been shown as a series of dots. The data for each year are based on the weights of 3000–4000 fruits.

It can be seen that in all 3 years the percentage of uniformly coloured fruits picked declined rapidly with increasing fruit weight until a very low level was reached at approximately 100 g. With further increase in size there was a tendency in two of the years for the percentage of uniformly coloured fruits to increase slightly.

From a consideration of the Grades A and C data the reasons for the shape of the curves for uniformly coloured fruits becomes apparent.

With increasing fruit size the percentages of Grade A fruits steadily increase until a maximum is reached between approximately 80 and 120 g. In 1955 the number of small fruits in Grade A was not sufficient to define the trend. Once the maximum had been reached the percentage declined with further increase in fruit size in two of the years, but in the third year remained constant.

For Grade C fruits, however, the percentage increased in all 3 years as the size increased, with no indication of an optimum.

Thus the initial rapid decline in the percentage of uniformly coloured fruits with increasing fruit size is due to the fairly steady increase in both Grades A and C. The check in the rate of decline at about 100 g. is caused by the fact that the optimal fruit size for the occurrence of Grade A lies between approximately 80 and 120 g. and the slight increase found in 2 years in the occurrence of uniformly coloured fruits with further increase in fruit size is due to the decline in the number of Grade A fruits partly offsetting the continued increase in Grade C fruits. It is of interest to note that the year in which the size trend for Grade A fruits was most pronounced was the year in which that for Grade C fruits was least pronounced, and vice versa. This antithetic relation between the two grades has been observed in other aspects of these two disorders.

It has been shown elsewhere (Cooper, 1959) that fruit size is not consistently correlated with either the length of the maturation period or the form of the growth curve of the fruit. It therefore follows that the occurrence of abnormal pigmentation

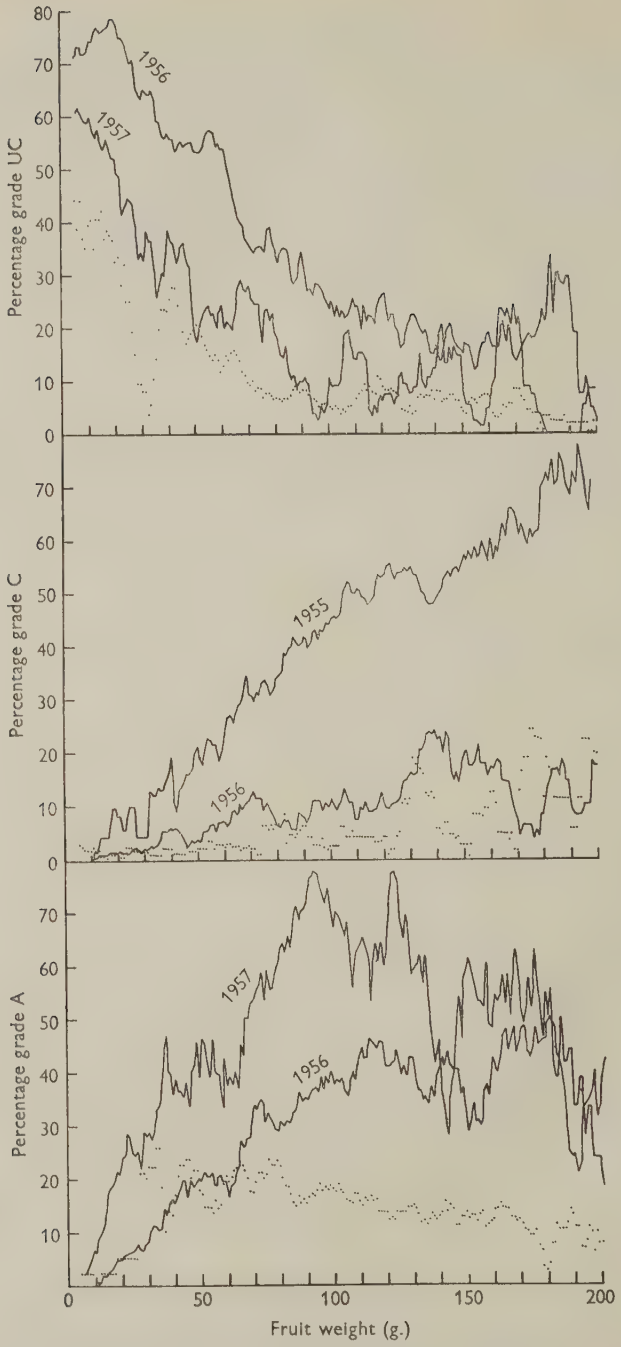

Fig. 1. Number of fruits in Grades A, C and UC expressed as percentage, at 1 g. intervals, of total number of fruits picked.

is not correlated with the length of the maturation period or the form of the growth curve of the fruit.

To check this deduction the intervals between flower opening and fruit picking (the maturation period) were recorded for individual fruits on selected plants, and the relation between the length of the maturation period and the types of pigmentation abnormality occurring was determined. Very small fruits (less than 20 g.) with a very long maturation period develop occasionally at the distal end of an inflorescence, and these fruits are usually uniformly coloured or show the Grade Z type of pigmentation. The mean maturation periods in days have therefore been shown in Table 1 both for all fruits and for those of more than 20 g.:

TABLE 1

	Grade UC	Grade A	Grade C	Grade Z
Fruits heavier than 20 g.	68	70	65	71
All fruits	92	70	65	88

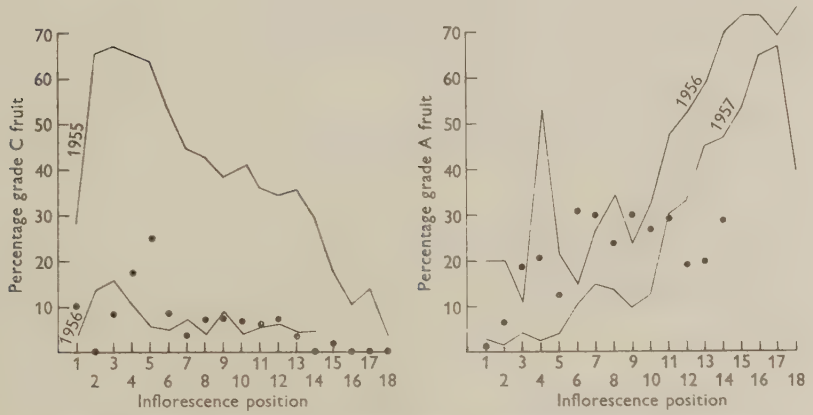

Fig. 2. Number of fruits in Grades A and C in each inflorescence expressed as percentages of total fruits in each inflorescence.

At the 5% level of probability there were no significant differences between the lengths of the mean maturation period even when the data for all fruits were used, thus confirming the previous deduction that the occurrence of pigmentation abnormalities is not correlated with the length of the maturation period.

In Fig. 2 the percentages of Grades A and C fruits occurring in each truss are shown. The values from the year in which the relation with truss position is least well defined are shown by solid circles.

Only small percentages of Grade A fruits occurred in the lower inflorescences, the proportion increasing with progress up the plant until quite large percentages occurred in the upper inflorescences.

Conversely, the greatest percentages of Grade C fruits occurred in the lower

inflorescences, excluding the first, and the proportion decreased with progress up the plant. The antithetic relation between Grades A and C referred to earlier is thus again demonstrated. It is also of interest to note in this connexion that again the year in which the trend for Grade A fruits was least well-defined (1955) was the year in which the trend for Grade C fruits was most clearly defined, and vice versa.

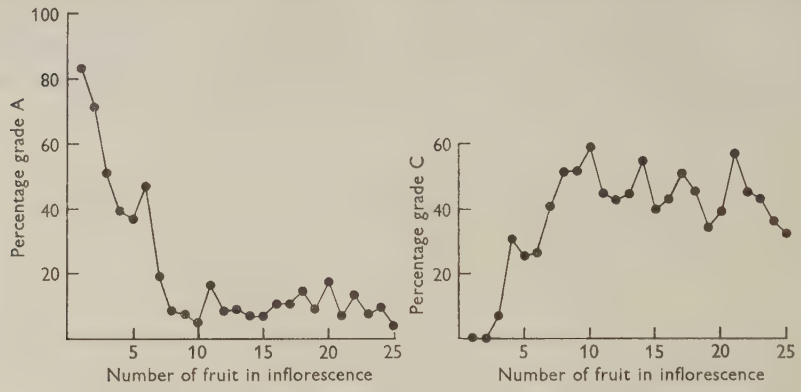

Fig. 3. Percentage of fruit in Grades A and C occurring in inflorescences of differing sizes.

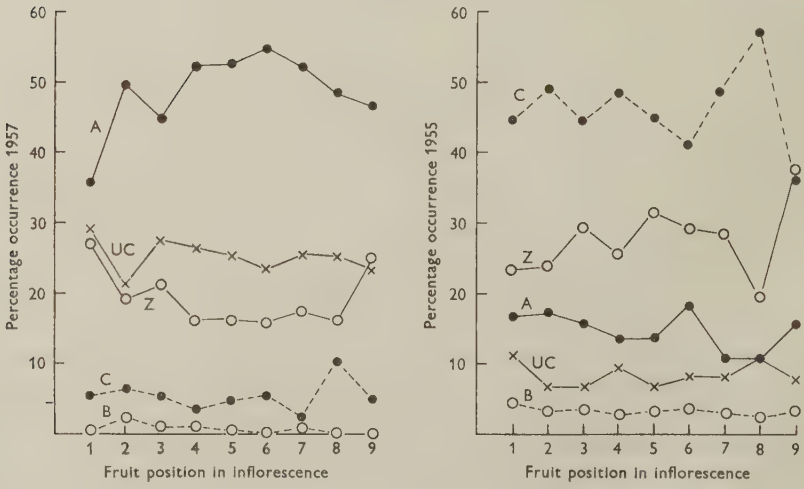

Fig. 4. Relation between pigmentation and fruit position in inflorescence.

There was no consistent relation between Grade Z and inflorescence position: the data were inadequate to test Grades B and D.

In none of the 3 years was there any effect of inflorescence size on the occurrence of pigmentation abnormalities of the B, D and Z type, and the occurrence of the

abnormalities was independent of the number of fruits that developed in an inflorescence. Similarly there was no consistent effect on the occurrence of Grades A and C fruits. It is of interest to note, however, that in 1955, the only year in which there was a relation with inflorescence size, the effect on Grade A fruits was, once more, the antithesis of that of Grade C fruits. In Fig. 3 it can be seen that when there were fewer than seven to eight fruits in an inflorescence the proportion of fruits with Grade A symptoms increased, whereas the proportion with Grade C symptoms decreased as the inflorescence size decreased.

The data on the influence of the position of the fruit within the inflorescence are shown in Fig. 4. In 1 year the data were not collected, and, consequently only 2 years' data are shown. It can be seen that in neither year was there any effect of position within the inflorescence on the occurrence of any type of pigmentation abnormality. It is again of interest to note another antithetic relation between Grades A and C. In 1957, when there were numerous Grade A fruits (48%), there were only a few Grade C fruits (5%), while in 1955 there were many Grade C fruits (46%) and few Grade A fruits (15%), suggesting that if in any one season the conditions are favourable to the occurrence of Grade A fruits they are not favourable to the occurrence of Grade C fruits, and vice versa.

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THE EFFECT OF CERTAIN SOIL TREATMENTS ON *DIDYMELLA* STEM-ROT OF TOMATOES

II. LABORATORY INVESTIGATIONS

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(With 1 Text-figure)

About 93 % of *Didymella lycopersici* spores were destroyed after 4 weeks incubation in unsterilized soil. A survey of the microflora of glasshouse soil receiving different treatments and inoculated with *D. lycopersici* showed no clear relation between numbers of any group of organisms and the incidence of stem rot. Sterilized soil was not made toxic to *D. lycopersici* by the growth of a number of soil micro-organisms even after 9 months incubation, but addition of unsterilized soil or of a suspension of unsterilized soil quickly restored toxicity. Direct observations of spores in soil on slides showed that their fate varied with the treatment of the soil before inoculation. With fresh soil or air-dry soil moistened 2 or more days before inoculation, lysis of spores occurred. With air-dry soil moistened and inoculated simultaneously, some spores germinated but growth of germ tubes soon ceased. No direct connexion could be seen between the fate of the spores and soil micro-organisms. Addition of glucose to unsterilized soil reduced its toxicity to *D. lycopersici*. Soils steamed for 1 min. or longer were not toxic to *D. lycopersici*, but soils steamed for very short periods were as toxic as unsterilized soils although the soil microflora was much reduced.

Glasshouse experiments (Williams, Sheard, Enid & Read, 1953; Williams & Hack, 1957) have established that *Didymella lycopersici* spores are effectively destroyed in unsterilized soils. Laboratory investigations to determine the rate of destruction and explore the nature of the factors concerned are here reported.

Methods

The soil used was from the glasshouse employed for field experiments (Williams & Hack, 1957). It was passed through a $\frac{1}{12}$ in. mesh sieve and kept moist at room temperature.

Plating methods conformed to the recommendations of Brierley, Jewson & Brierley (1927–8) except that shaking was sometimes done by machine.

Bacteria and actinomycetes were counted on plates of the soil extract medium 'YS' of Lochhead & Chase (1943). A medium devised by Sleeth (1945) was modified for fungi and *D. lycopersici*, glucose being decreased to 0·25 or 0·5 %, and 0·0067 % rose bengal added. Growth on this medium was slow, giving an opportunity for many fungus and *D. lycopersici* colonies to develop, an advantage when only a small amount of *D. lycopersici* was present and dilutions had to be low. Plates were incubated in the dark, as exposure to light was found to result in the elimination of

many of the colonies. When counting *D. lycopersici* in crowded plates, any doubtful colonies were picked off and inoculated into green tomato fruits where characteristic lesions with pycnidia developed after 1–2 weeks. Incubation temperatures were 21° C. for bacteria and 16–17° C. for fungi, the low temperature favouring the growth of *D. lycopersici* more than that of many of its competitors.

For direct microscopic observations of the fungus in the soil 'soil slides' were used (Hack, 1957). In some cases, in order to avoid the sterilizing effect of air-drying, moist soil was shaken on to the agar-coated slides through a $\frac{1}{40}$ in. mesh sieve. Soil for this purpose was dried to a tension of 36 cm. Hg on a tension table controlled by a manostat modelled on those of Jamison & Reed (1949). Such 'fresh soil' slides were further moistened after the sprinkling process and thereafter treated as the air-dry soil slides.

To study the effect of different soil treatments on *D. lycopersici* known numbers of spores were added in 100-ml. Erlenmeyer flasks to 90 g. sieved soil at 30% moisture content calculated on an oven-dry basis. The flasks were incubated for different periods at room temperature either in a saturated atmosphere or with additions of distilled water as necessary, and plated. In some experiments spores were added to soil slides which were similarly incubated and plated.

Results

(1) *Rate of disappearance of* Didymella lycopersici *spores*

To study the percentage of *D. lycopersici* spores surviving in unsterilized soil at successive weekly or fortnightly intervals after inoculation, many spores were added to soil in flasks or on soil slides and three replicates were plated immediately and three after each incubation interval. In ten experiments, from 1·6 to 23% of added spores survived 4 weeks incubation; the mean was 7·1%. The proportion of spores dying per unit time remained fairly constant for 6 weeks (Fig. 1).

The slow disappearance of fungus spores added to unsterilized soil has been reported by several workers; for example, Katznelson (1940) reported survival of 21·6% of added *Fusarium culmorum* spores after 32 days and 25·5% of *Penicillium* spores after 20 days (see also, Park, 1955; Rishbeth, 1955; Rombouts, 1953).

(2) *Microflora of glasshouse soils after treatment and incidence of* Didymella *stem-rot*

Surveys of the soil microflora were carried out during the 3 years of the glasshouse experiments, in most detail during 1954, when samples were taken from three of the four replicate plots of treatments on six occasions during the season. The treatments sampled, and the numbers given in Table 1, were *2* (soil treated with 2% formalin, inoculated), *3* (untreated), *5* (steamed, stable manure equivalent to 60 tons per acre dug in before steaming, inoculated), *6* (steamed, inoculated), and *8* (steamed, 1½ barrow loads maiden loam pricked into the surface layers before inoculation). The numbers of bacteria, actinomycetes and fungi at the time of inoculation with *Didymella lycopersici* spores (21 December 1953) and 10 days after

TABLE 1. *Numbers of soil organisms in glasshouse soil, means for three plots, and incidence* Didymella *stem-rot*

Treatment	21 December 1953			18 March 1954			Mean no. of plants with basal lesions. 20 plants per plot
	Bacteria	Actino-mycetes	Fungi	Bacteria	Actino-mycetes	Fungi	
2. Formalin, inoculated	$12{\cdot}6\times10^{7}$, *3*, 5, *6**	$6{\cdot}7\times10^{5}$, 3	$1{\cdot}7\times10^{3}$, **3**, 6, **8**	$43{\cdot}0\times10^{7}$, 8	211×10^{6}, 5	$46{\cdot}0\times10^{3}$, **3**, 5	$6{\cdot}5\pm1{\cdot}04$
3. Untreated	$3{\cdot}5\times10^{7}$, *2*	$12{\cdot}2\times10^{5}$, 2	$53{\cdot}2\times10^{3}$, **2**, **5**, **6**	$42{\cdot}7\times10^{7}$, *8*	244×10^{5}, **5**	$114{\cdot}0\times10^{3}$, **2**, 5, **6**, 8	0·75
5. Steamed + 60 tons/acre stable manure, inoculated	$6{\cdot}1\times10^{7}$, 2	$2{\cdot}2\times10^{5}$	$3{\cdot}7\times10^{3}$, **3**, 8	$39{\cdot}3\times10^{7}$, **8**	523×10^{5}, **3**	$16{\cdot}2\times10^{3}$, 2, *3*, 8	$12{\cdot}25\pm0{\cdot}85$
6. Steamed, inoculated	$5{\cdot}4\times10^{7}$, *2*	—	$5{\cdot}6\times10^{3}$, 2, **3**, 8	$36{\cdot}7\times10^{7}$	167×10^{5}	$16{\cdot}8\times10^{3}$, **3**	$14{\cdot}0\pm1{\cdot}08$
8. Steamed, maiden loam pricked in, inoculated	$9{\cdot}2\times10^{7}$	$2{\cdot}2\times10^{5}$	$46{\cdot}3\times10^{3}$, **2**, 5, 6	$20{\cdot}1\times10^{7}$, 2, *3*, **5**	55×10^{5}	$56{\cdot}4\times10^{3}$, 3, 5	$9{\cdot}5\pm0{\cdot}86$

* Numbers refer to treatments from which each value differs significantly (*t* tests)
Those in heavy type differ at the 1 % level.
Those in italics differ at the 0·1 % level.
The others differ at the 2 or 5 % level.

the tomatoes were planted, together with the number of plants showing basal lesions during the experiment, are given in Table 1.

Plots receiving treatments *5*, *6* and *8* were steamed on 26 November 1953. Formalin was applied to treatment *2* plots on 2 December and all were flooded on 11 December. Thus, on 21 December there had been time for considerable recovery of soil micro-organisms from the effects of the sterilizing treatments. Actinomycetes and fungi were fewer than in the untreated plots but bacteria were more numerous. In March, bacteria and actinomycetes were very numerous in all plots. Numbers of fungi in treated plots were still low compared with untreated plots, but were more numerous in the formaldehyde and maiden loam plots (treatments *2* and *8*) than in the steamed, and basal lesions were fewer in these plots than on the steamed plots.

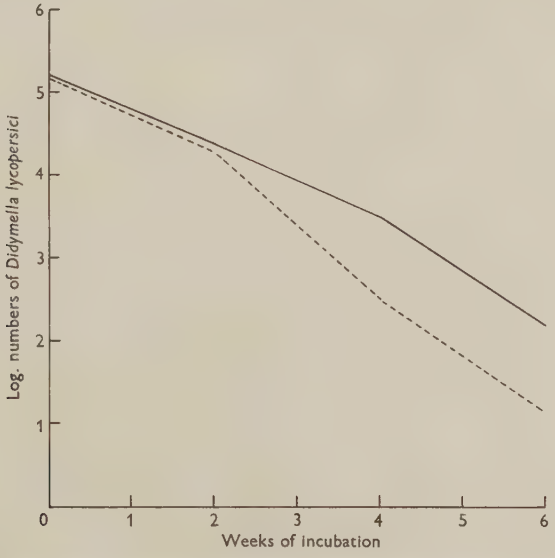

Fig. 1. Numbers of *Didymella lycopersici* spores present during incubation in unsterilized soil. ——, Spores in flasks; - - - - -, spores on soil slides.

(3) *Effect of re-inoculation of autoclaved soils on* Didymella lycopersici

(*a*) *Re-inoculated soils not incubated before addition of* Didymella lycopersici

Several actinomycetes and fungi added to autoclaved soil with *D. lycopersici* failed to decrease spore survival. Soil suspensions filtered through Whatman no. 1 filter-paper added to autoclaved soil reduced spore survival, and these suspensions contained mostly bacteria (Williams & Hack, 1957). Bacteria isolated at random from platings of these suspensions were tested alone and in groups for effectiveness against *D. lycopersici* but only occasionally was any action found. Results of such an experiment are shown in Table 2.

Hack (1957) showed that there is a reduction in numbers obtained by plating as a result of spore germination in the soil. From her results it may be surmised that where 60–80% of the original numbers can be accounted for, spore germination has taken place and the fungus has made some growth in the soil but has not sporulated again. The very large number of bacteria present in treatment *8* may have prevented spore germination, so that numbers found on plating were the same as before incubation.

TABLE 2. *Amount of* Didymella lycopersici *in re-inoculated, autoclaved soil in flasks after* 4 *weeks incubation* (*three replicates*)

Treatment	% original
1. Autoclaved	66·0
2. Unfiltered 10% soil suspension shaken 20 min.	8·8*
3. Suspension filtered once through Whatman No. 1 filter paper	29·3*
4. Bacterium *a*	77·7
5. Bacterium *b*	75·9
6. Suspension of 2 species of bacteria	69·9
7. Suspension of 3 species of bacteria	77·4
8. Suspension of 5 species of bacteria	103·9*

* Significantly different from Treatment 1.

(*b*) *Re-inoculated soils incubated before addition of* Didymella lycopersici

Glasshouse soils steamed in the autumn are, by the autumn of the following year, once more as toxic to *D. lycopersici* as unsteamed soils. This toxicity might be due not to specific antibiotic organisms, but to the effect of a long period of activity in the soil by the microflora. Accordingly, flasks containing 25 g. of soil were autoclaved and inoculated with different soil organisms, singly or in combination. Other similar flasks were inoculated with unsterilized soil or a suspension of unsterilized soil. All the flasks were incubated for periods up to 9 months before inoculation with known numbers of spores of *D. lycopersici*. Four weeks after inoculation with *D. lycopersici* they were plated. None of the soils inoculated with soil organisms developed the power to destroy *D. lycopersici* spores, even after the lapse of 9 months. Where, however, soils had been inoculated with 1% unsterilized soil, or 1 ml. of a 10% suspension of unsterilized soil, spores of *D. lycopersici* were always destroyed. For example, after 8 months incubation, 16·7% of the original number of spores were left after 4 weeks in soil inoculated with soil suspension, 13·5% where 1% unsterilized soil had been added, and 4% in unsterilized soil.

(4) *Direct observations of* Didymella lycopersici *in soil*

Observations of the disintegration of fungus spores in unsterilized soil, either with or without germination, have been reported several times (Dobbs & Hinson, 1953; Park, 1955).

The appearance of *D. lycopersici* spores added to unsterilized soil slides depended on whether or not the air-dry soil had been moistened and incubated before they were added. When slides were prepared with fresh soil or with air-dry soil

moistened and incubated 2 or more days before inoculation, *D. lycopersici* spores showed no change except that some developed a light-brown colour. It became more and more difficult to find spores during the 6 weeks after inoculation, though there was no indication of the cause of their disappearance. Bacteria developed profusely and fungi sporadically. Algae grew copiously on slides made with fresh soil but not where air-dry soil was used. Protozoa were numerous in the film of water over the slides. None of these groups of organisms, however, seemed to be associated in any special manner with the spores. When the air-dry slides were moistened and inoculated simultaneously, up to 30% germination occurred, and a high proportion of the remaining spores swelled. Activity was further manifested by the development of brown pigment in many germinated and ungerminated spores, but no further development occurred. Plate counts of viable spores present after 1 month showed no differences between slides which had been incubated before inoculation in a moist condition and slides which had not, in spite of their different appearances.

Spores of *D. lycopersici*, when added to soil slides that had been autoclaved, swelled and germinated, mycelium developed over the whole slide, and pycnidia with spores appeared, usually about 2 weeks after inoculation. Brown pigment developed as in unsterilized soil, and often appeared to diffuse through the cell walls, forming a halo around them. This browning appears to be a reaction of the live fungus to soil and begins about 2 days after exposure to it. It does not occur when heat-killed spores are added to soil slides, or when spores are added to 'sand slides' made up like soil slides. The browning is distinct from the carbonized hyphae which form in old cultures of *D. lycopersici*.

Spores of *D. lycopersici* on slides autoclaved and re-inoculated with bacteria behaved like those in autoclaved soil. Pycnidia developed on some, but when growth of bacteria was very active, growth of *D. lycopersici* was sometimes suppressed.

TABLE 3. *Numbers per g. of moist soil of micro-organisms* 8 *weeks after the addition of glucose and* % Didymella lycopersici *surviving* 4 *weeks incubation*

Glucose	Bacteria	Actinomycetes	Fungi	*D. lycopersici* % original no.
None	$1{\cdot}16 \times 10^7$	$1{\cdot}04 \times 10^7$	$4{\cdot}30 \times 10^4$	15·8
1%	$19{\cdot}6 \times 10^7$	$10{\cdot}7 \times 10^7$	$22{\cdot}7 \times 10^4$	18·3
5%	188×10^7	$28{\cdot}6 \times 10^7$	585×10^4	66·4*

* Significantly different from 0 and 1% glucose which do not differ from each other.

(5) *Effect of adding glucose to soil on toxicity towards* Didymella lycopersici

West & Hildebrand (1941) have shown that addition of carbohydrate alters the bacterial-balance index and fungus composition of soils, and Dobbs & Hinson (1953) that it destroys inhibition of fungus spore germination. The effect on *Didymella lycopersici* was therefore investigated.

1 and 5% by weight of powdered glucose were added to 500 g. lots of soil and incubated in the laboratory for 1 month with 500 g. soil without glucose. Four

25 g. portions were weighed out from each lot and all were inoculated in flasks with 1 ml. of spore suspension. One flask of each treatment was plated immediately; the rest were plated after 4 weeks incubation (Table 3).

Whether spores germinated or not in the soils with added glucose is not known, but the presence of such a large microflora makes it unlikely. Glucose improved this soil as a medium for fungi; not only was there a massive increase in their number soon after its addition, but after a month the soil was not so toxic to *D. lycopersici* as unamended soil. These results contrast with those of Park (1955) who found all stimulation of added fungi by 3% glucose was over in 5 days, and numbers were fewer than in unamended soil by 8 days.

(6) *Effect of heating soil for very short periods on toxicity towards* Didymella lycopersici

By steaming soil for less than the 10 min. of normal glasshouse practice a series of microfloras was produced and tested for destruction of *D. lycopersici*, the aim being to kill off selectively more main groups of micro-organisms with each lengthening of the heating period. In early experiments, steam from an autoclave

TABLE 4. *Numbers per g. of moist soil of micro-organisms in heat-treated soils and* % Didymella lycopersici *surviving 4 weeks incubation*

Organisms	Unheated	Heated to 100° C. Withdrawn immediately	Heated at 100°C. for 1 min
% original no. *D. lycopersici* after incubation	20·8	30·3	81·1
Fungi: Before incubation	380×10^2	$5{\cdot}97 \times 10^2$	0
After incubation	244×10^2	$60{\cdot}3 \times 10^2$	0
Bacteria: Before incubation	151×10^5	$65{\cdot}6 \times 10^5$	$0{\cdot}3 \times 10^5$
After incubation	307×10^5	$10{,}700 \times 10^5$	102×10^5
Actinomycetes: Before incubation	$5{\cdot}2 \times 10^6$	$0{\cdot}12 \times 10^6$	0
After incubation	$1{\cdot}1 \times 10^6$	$10{\cdot}6 \times 10^6$	0

at 30 lb. pressure was passed into soil contained in a box, in the bottom of which was a perforated grid similar to these used in commercial practice. Later, a small copper coil pierced with holes which fitted into a 500 ml. beaker was used. The first sample was taken when the temperature of the soil reached 100° C. Further samples were taken at intervals thereafter. Four 25 g. portions of each sample were weighed as aseptically as possible into 100 ml. flasks and inoculated with spores of *D. lycopersici*. One flask of each heating period was plated immediately, the remainder after 4 weeks incubation.

Different steaming intervals were tested in five trials. *D. lycopersici* spores were not destroyed when added to soils maintained at 100° C. for 1 min. or longer, but those added to soil heated to 100° C. and immediately withdrawn were destroyed to the same, or even greater, extent as spores added to unheated soil. Numbers of bacteria, actinomycetes and fungi were greatly reduced by this very short heating. Bacteria and actinomycetes increased to more than pre-steaming levels during the

month's incubation, whereas fungi increased but did not reach pre-steaming levels. Protozoa survived the very short heat treatment (direct observation of small quantities of soil in water), but not when heating lasted 1 min. or more. Fungi were eliminated by heating for 1 min. or longer and bacteria and actinomycetes were reduced more and more as the steaming interval lengthened. During incubation they increased, but in contrast to soil withdrawn on reaching 100° C., remained fewer than in unsteamed soil. Data from a typical experiment are given in Table 4.

Discussion

When *D. lycopersici* spores are kept in distilled water for 4 weeks too crowded to germinate, an insignificant number die. Partially germinated spores also do not suffer in 4 weeks on soil slides autoclaved, but so heavily contaminated with bacteria that growth is suppressed. It seems, therefore, that it is not ageing or starvation which destroys *D. lycopersici* spores in unsterilized soils, and the present experiments were guided by the hypothesis that biological agents are responsible.

No particular organism, or even group of organisms, has been shown to be related to the disappearance of *D. lycopersici*. Fungi are decreased by steaming more than bacteria, and they fail to recover to pre-steaming levels until late in the season, if at all (Williams & Ebben, 1952). Their numbers were also relatively high in the formalin and maiden-loam experiments of 1954 (Table 1) in which the incidence of the disease was much less than in steamed soil. These facts suggest that they may play a part in the destruction of *D. lycopersici*. But fungus numbers in contrast to those of bacteria and actinomycetes are low in the neutral soil in question, and experimental work has shown that there is no marked qualitative shift following steaming. Furthermore, in experiments reported in §§3*a* and 6 above, destruction of *D. lycopersici* occurred where fungus numbers were much reduced by filtering and heating and *D. lycopersici* survived despite very high numbers of fungi, bacteria and actinomycetes in soil with added glucose.

Bacteria are stimulated in number by steaming and actinomycetes do not remain depressed as long as fungi. However, different bacteria and actinomycetes may be dominant before and after steaming (Anon. 1956). Protozoa have always been found present where *D. lycopersici* spores are destroyed, and absent in conditions where they survive; but their numbers and importance appear small compared with the other main groups of the soil microflora.

Efforts were made to determine whether the unknown biological agents produce substances effective in the soil solution or not. Soil solution was obtained by the alcohol-displacement method (Parker, 1921), by centrifugation and by suction through the walls of an L7 filter candle inserted in the soil, but not even the slight antifungal action obtained by Jefferys & Hemming (1953) was found. The slightly nutritive extracts proved, in fact, excellent for spore germination and good for some growth. An explanation for these failures might be that the destructive agency is mainly located on the soil particles or other solid material (for example, developing on the glass between soil particles on soil slides). Park (1956, 1957*a*, *b*) has in fact found that the lysis of various fungi in the presence of other organisms

occurs in the presence of sand and soil to a much greater extent than in agar or liquid media, and suggests the increased toxicity is a stereotactic phenomenon. Alternatively, the inhibiting substance may be readily destroyed, possibly by oxidation, and be active only in the presence of the organism or organisms which produce it.

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DIDYMELLA STEM-ROT OF OUTDOOR TOMATOES

I. STUDIES ON SOURCES OF INFECTION AND THEIR ELIMINATION

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In studies on *Didymella* stem-rot of outdoor tomatoes several possible modes of introduction of the disease were investigated, together with methods of avoiding them. The pathogen was found to invest and penetrate the seeds in diseased fruit without destroying their viability and methods were devised of ridding the seeds of infection. It was not, however, possible to demonstrate the infection of mature plants from infected seed. It was found that the disease could be introduced on contaminated canes, in potting soil and from plant debris from the previous season's crop. The spread of the disease was encouraged by overhead irrigation.

Tests of strains of various species of *Lycopersicon* showed that one line of *L. hirsutum* was highly resistant to stem and root infection although another line was highly susceptible.

Didymella stem-rot of tomato appears to have been first recognized in Britain in 1885 (Moore, 1949*a*, *b*) and it is known in many European countries. It is particularly prevalent on outdoor tomatoes in Jersey where it has been studied by Small (1940) and later by Phillips (1956*a*, *b*). In England, crop losses have fluctuated considerably during the past 50 years. Between 1906 and 1909 much damage was caused in the Lea Valley (Moore, 1949*b*) and the disease was very severe during both world wars (Williams, Sheard & Read, 1953). It became very widespread from 1939 to 1945, coinciding with the increase in the acreage of outdoor tomatoes during this period from 229 to 4537 acres. Since 1945 there has been a sharp decline in the cultivation of outdoor tomatoes with a corresponding reduction in the extent of the disease but the crop has remained of importance in some areas, notably in south-east England. By 1952 it was felt by officers of the N.A.A.S. and others that more information was necessary on the etiology of the disease and on possible control measures. The present investigation was therefore started in 1953 under an Agricultural Research Council grant. The study of the disease has been divided into five sections: (*a*) sources of disease and methods for their elimination; (*b*) spread of infection; (*c*) tests of *Lycopersicon* species for resistance; (*d*) observations on the pathogen; and (*e*) chemical control. This paper deals with the first three of these sections, the work on the pathogen and on chemical control forming the subject of other papers.

* Including part of a thesis by this author approved for the degree of Doctor of Philosophy of the University of London.

METHODS

The fungal isolate used in all infection tests, except where otherwise stated, was obtained by Dr D. H. Phillips from a stem lesion in Jersey in 1952. In the present work it proved to be extremely virulent toward both stems and fruits and did not lose pathogenicity during repeated subculturing. In 1953–4 the fungus was cultured on tomato juice agar (100 g. ripe tomato fruit sliced and boiled for 5 min. in 500 ml. water, filtered and made up to 1 l.; 25 g. agar added). In 1955–6 a modified Coon's agar was used (maltose 7 g., KNO_3 2 g., KH_2PO_2 2·7 g., $MgSO_4$ 1·2 g., agar 20 g., water 1 l.). Pycnospores were plentifully produced on both media after 9 days growth at 20° C.

For the 1953 experiments tomato plants cv. Radio were grown from seed obtained from an outside source. In 1954–7 plants of cv. Harbinger were used, the seed for most of which was obtained from fruit on plants specially raised in isolation and free from visible infection with *Didymella*. Experimental plants were raised in glasshouses and cold frames until 5–7 weeks old, according to normal commercial practice, being then planted in the field or repotted for glasshouse tests.

In the field the plants were usually tied to canes and grown, for ease of handling, 3 ft. apart in rows 5 ft. wide. This is considerably wider than normal commercial spacing.

In infection tests, *D. lycopersici* was identified by the presence of typical lesions bearing pycnidia which ejected a proportion of bicellular spores.

SOURCES OF DISEASE AND METHODS OF ELIMINATION

Infected seed

Previous evidence on the importance of infected seed as a source of disease in the crop is extremely conflicting. Schoevers (1929) and Sheard (1944) found no infection in plants grown for 4 months from infected seed. Phillips (1956*a*), growing plants under conditions where they were exposed to heavy external infection, found no significant difference in the amount of disease occurring in those raised from diseased and clean seed, although the disease appeared 3 weeks earlier on the former plants. On the other hand, Ogilvie (1945) reported infection of seedlings grown from diseased seed under moist conditions and Scott (1955) obtained 44% infection in starved plants raised from seed artificially infected with spore suspension and 1·3% infection in similar plants raised normally. Hickman (1946) obtained heavy infestation (up to 30%) in plants grown from naturally infected seed but no control plants were grown from clean seed in this experiment.

In view of these conflicting results and the importance of the introduction of even a very few infected plants into a previously clean nursery a special study was made of this subject by the writers.

Histological studies. Orth (1939) first identified hyphae and pycnidia on the surface and beneath the seed coat of infected seed. He stated that infection occurred through the seed coat and not through the placenta and funicle.

In the present work, sections were made of diseased fruit (cv. Stonor's Money-Maker) bearing black lesions ½–1 in. diam. at the calyx end. Hyphae and pycnidia were present in the placenta, within the funicle and surrounding many seeds. Sections of freshly extracted seed showed that hyphae and pycnidia occurred within the seed coat, from the funicle to the distal end of the seed and mainly within the gaps between the cells of the middle region of the coat. None was seen in the single layer of cells comprising the inner coat or in the endosperm or embryo (Fisher, 1954). Isolates of *Didymella* from pycnidia dissected from dried seed were pathogenic to tomato stems. Artificial infections of tomato fruit showed the progressive growth of the fungus down the placenta and into the seeds via the funicle during a period of about 30 days. The observations of Orth (1939) on the site of infection were thus confirmed but not his interpretation of the mode of penetration of the seed.

Estimation of seed infection. Some previous workers have estimated the numbers of infected seeds in a sample by germinating them on sand or filter-paper and counting those bearing pycnidia on the radicle, hypocotyl or cotyledons. Others have placed the seeds on agar media without previous surface sterilization and observed the growth of *Didymella* from them. When used by the authors the first method gave inconsistent results and in the second method the growth of contaminants obscured the growth of *Didymella*. Estimations of total infection were therefore made by using green tomato fruits as a selective medium. These were swabbed with alcohol and placed individually (on glass supports) in sterile tins each containing 2 ml. of sterile water. A drop of sterile water was placed on the calyx scar of each fruit together with one seed and the tins were closed by tight-fitting lids and kept at room temperature for 4 weeks. Twenty seeds were selected for each test by the random cup method (*International rules for seed testing*, 1953). The pathogen grew out from infected seeds and entered the fruit, producing hyphae and pycnidia in the placenta, other fungi remaining on the surface of the fruit.

This technique is very suitable for experimental work but probably not for large-scale testing, owing to the number of fruits required and their unavailability at certain times of the year.

For the determination of internal seed infection the seeds were dipped for 30 sec. in 70% alcohol and then for 10 min. in 3% chlorine solution (27% Chloros) (Groves & Skolko, 1944). The seeds were then drained on filter-paper and plated on drops of Coon's agar, ten per Petri dish. Infection was assessed after 7 days. The results of the determinations of total and of internal infection are shown in Table 1. Several workers have reported that seed loses its infection after 1 year's storage (e.g. Phillips, 1956*a*). As shown in Table 1, the fungus was obtained from seeds from various sources that had been stored for periods of from 20 to 44 months. On the other hand, none of the seeds tested showed internal infection after 12 months storage except for the sample of Smooth Skin which did so after 21 months but not after 44 months.

Fifty seeds from a sample that had shown 2·5% internal infection in the surface-sterilization test after 3 months storage were examined for the presence of hyphae and pycnidia within the seeds. The results are shown in Table 2.

It is apparent that the percentage of seeds with visible internal infection was much greater than the 2·5% indicated by the surface sterilization test. It is unknown whether the treatment killed the fungus in some of the seeds or whether it was non-viable.

Table 1 shows that many of the infected seeds were capable of germination, even after long storage. The sample on which the assessment of internal infection was made by microscopic examination had a germination capacity of 98%. The presence of the fungus in 22% of the seeds thus did not affect their germination. This was tested both on filter-paper in Petri dishes and in boxes of soil, with comparable results.

TABLE 1. *Percentage germination and infection of seed extracted from tomato fruit infected with* Didymella lycopersici

Cultivar	Source of fruit	Year of extraction	Year of test	Age (months)	Germination (%)	Total infection (%)	Internal infection (%)
Harbinger	N.V.R.S. (healthy)	1955	1956	8	97	0	0
Harbinger	N.V.R.S.	1955	1956	8	98	55	12·5
Harbinger	Sussex	1953	1954	8	—	—	5
Harbinger	Alcester, Warwickshire	1954	1956	20	46	75	0
Harbinger	Angmering, Sussex	1953	1956	32	84	50	0
Smooth skin	Chichester, Sussex	1952	1954	21	—	—	5
Smooth skin	Chichester, Sussex	1952	1956	44	46	55	0
Money-maker	Commercial seed	1951	1954	36	—	40	—

TABLE 2. *Microscopic examination of infected seed*

(Percentage of seeds bearing)

Hyphae and pycnidia		Hyphae only		
External	Internal	External	Internal	No fungus
22	18	46	4	32

Infection of plants from seed. Ogilvie (1945) observed that naturally infected seed, when germinated in soil and grown under moist air conditions, in two tests gave 5 and 8·5% of diseased seedlings. In contrast, Scott (1955) found 3% of infection in seedlings grown from naturally infected seed under dry conditions but no infection when they were grown in humidity chambers.

In the present studies such seedlings grown on filter-paper in moist dishes have frequently borne pycnidia and hyphae but when similar infected seed was sown in autoclaved, steamed or unsterile soil under dry or saturated conditions no infection was found.

Ogilvie (1945) suggested that infection of young plants might occur through the cotyledons if the infected seedcoats remained attached to them. Although this has

not been seen by the writers it has been possible to induce lesions on the stems of young seedlings grown under humid conditions, by inoculating the tip of a cotyledon with an agar culture of the fungus.

Several experiments have been made on the amount of infection occurring among plants grown to maturity from infected seed. In all these tests the plants were raised under normal, fairly dry, glasshouse conditions.

In single-plot trials during 1954 on sources of infection a plot containing fifty plants, grown from seed showing 25% total and 5% internal infection, eventually contained 42% of infected plants. The control plot showed only 8% infection, but the comparison between the plots was invalidated by the presence of other very heavily infected plots.

In 1955 a replicated randomized trial, isolated from external infection, was made in which 165 plants were grown from seed with 25% infection and 105 plants from clean seed. No infection was seen in any of the plants but it was thought that this might have been because of the very hot dry weather. Three similar trials were made in 1956, which was cold and wet and very suitable for the development of the disease. In these a total of 315 plants was raised from four samples containing 50–75% infected seeds. One of these samples contained 12·5% of internally infected seeds. 180 plants were also raised from clean seed. The plants were grown in the field for 3 months but no infection appeared on any of them. It must be concluded that in this experiment the disease was not transmitted from the seed to the mature plants.

Seed disinfection

Numerous studies have been made of methods of ridding the seed of infection, in case further tests on seed transmission should indicate this to be advisable. The effects of the treatments on the fungus and on seed germination have usually been determined separately and suitable methods have been tested using infected seeds.

Tomato seed is sometimes extracted by fermenting the fruit in water. It is probable that some external infection of clean seed from fungus present on other seed or fruit may occur in this process. In other extraction methods hydrochloric acid or washing soda is used. It was found that 1·5% hydrochloric acid and 20% washing soda were lethal to the fungus, but seed extracted from diseased fruit using these compounds still contained respectively 10 and 30% of infected seeds. This may have been due to inadequate contact between the compound and the seed, or to the presence of internal infection. Trials were therefore made of heat treatment and of other chemical steeps.

Heat treatment. Van Koots & Brons (1949) reported that immersion of infected seed for 2 hr. in water at 47·5° C. would kill spores within pycnidia but slightly reduced seed germination.

In the present study the fungus was grown on bamboo chips which were then kept for 1 week in air at 60% R.H. (over 33·5% sulphuric acid). This treatment caused the chips to attain approximately the same moisture content as dry seed. Using these inoculated chips it was found that the fungus would withstand dry

heat for 1 hr. at 60° C. If the fungus was grown for 4 weeks continuously in an atmosphere of 60% R.H. it survived dry heat for 1 hr. at 75° C. but not at 80° C. If the chips were heated in water (after a preliminary 3 hr. soak) the fungus survived on all of them after heating for 1 hr. at 60° C. and on 10% of them at 65° C. A temperature of 70° C. was lethal. The results of heat tests on the germination of clean and infected seed (55% total and 12·5% internal infection) are given in Table 3*a* and *b*.

TABLE 3*a*. *Dry-heat treatment of tomato seed* (*cv. Harbinger*)

Type of seed	Germination (%)	Treatment time (min.)	% germination after treatment 65° C.	67·5° C.	70° C.	75° C.	80° C.
Clean	98	30	99	99	86	52	0
		60	98	98	78	28	0
Infected	88	30	66	50	15	3	0
		60	69	51	5	2	0

TABLE 3*b*. *Hot-water treatment of tomato seed* (*cv. Harbinger*)

Type of seed	Germination (%)	Treatment time (min.)	% germination after treatment 50° C.	52·5° C.	55° C.	60° C.
Clean seed	98	30	100	98	98	43
		60	99	100	65	0
Infected seed	97	30	77	76	58	0
		60	75	68	39	2

A comparison of the results in Table 3 with those given for the heat treatment of the fungus shows that germination (especially of infected seeds) was markedly reduced at temperatures below those necessary to kill the fungus.

Further tests in which infected seed was heated to 70° C. (dry) and 55° C. (in hot water) showed that a high proportion of the seeds remained infected.

A preliminary trial in which seeds and cultures of *Didymella* on bamboo chips were immersed in carbon-tetrachloride and heated showed that the fungus was then killed by treatment at 65° C. for 1 hr. whereas seed germination was only slightly reduced. It was not possible to test the effects of this treatment on the fungus in infected seed but the results indicate that the method would be worth further trial.

Chemical treatment. In the course of other studies, fifty-seven compounds were tested for toxicity to cultures of *D. lycopersici* growing on agar. Those organo-mercurial compounds which were fungicidal at low concentrations were then tested for their value as seed steeps by determining their effect on seed germination and on the fungus in infected seeds. It was found that when seeds showing 80% infection were steeped for 1 hr. in the following three compounds the fungus was killed while germination was unaffected: 0·001% ethyl mercuric phosphate; 0·25% Panogen and 0·66% Aretan. Phenyl mercuric nitrate (0·01%) and phenyl mercuric urea (0·003%) also killed the fungus *in vitro* and did not impair germination of the seed. It is thus apparent that tomato seed can be freed from *Didymella* infection by treatment with these compounds.

Field debris

In outdoor tomato plantations that are infected with *Didymella* there is often a large quantity of plant debris lying on the ground toward the end of the season. Fruits infected with the disease readily fall from the plants and may be trodden underfoot, and leaves and sideshoots are sometimes dropped on the ground. Complete clearing of such debris is difficult and consequently it is frequently ploughed in after cropping, although the haulm is usually removed. An experiment was conducted during 1953–4 to test whether such debris would give rise to infection in succeeding tomato crops.

In October 1953 infected haulm from a commercial nursery was buried 4 in. deep in trenches 1 ft. wide using 1 lb. of haulm per yd. of row. There were duplicate treated and control plots each containing 25 yd. of row. In June 1954 twenty-five 6-week-old tomatoes were planted in each plot. By late September fifteen of the fifty plants on the plots receiving the haulm bore basal lesions of *Didymella*, whereas only one plant of those on the untreated plots became infected. Infected plants were removed when they contracted the disease and the remaining plants were pulled up and burned at the end of the season. Similar plantings were made in 1955 and 1956 on the same site but no disease was seen.

In an adjacent area infected tomato fruits were buried in duplicate plots of the same size as those in the haulm experiment. The fruit was put in at two depths and at three rates. The treatments given and the amounts of disease recorded in the three successive years are shown in Table 4.

TABLE 4. *Percentage infection of tomatoes planted over diseased fruit*

	Infected fruit				Healthy fruit,	
	4 in. deep			9 in. deep,	4 in. deep,	No
Year	3 lb./yd.	1 lb./yd.	½ lb./yd.	1 lb./yd.	3 lb./yd.	fruit
1954	56	42	16	8	12	12
1955	2	2	0	0	0	0
1956	0	0	0	0	0	0

The high infection in the control plots invalidates the results obtained by burying 1 lb. fruit at 9 in. or ½ lb. at 4 in., but it is clear that 1 and 3 lb. of infected fruit buried 4 in. deep gave rise to heavy infection in 1954. There appears to have been some residual inoculum in these plots in 1955 but this result is of doubtful significance.

The results, which are in agreement with those obtained by Phillips (1956*b*), suggest that a 2-year rotation of tomato crops may give adequate control of soil-borne infection.

Potting soil

Conflicting reports occur in the literature on the infection of tomato plants from propagating soil. Orth (1939) was unable to induce infection by raising tomato seedlings in either naturally or artificially infected soil, and similarly, Oyler & Read

(1942) found that few plants contracted the disease from contaminated soil. Later, however, these workers (Williams *et al.* 1953) obtained infection of plants raised in both sterile and unsterile inoculated potting soil, there being more infection in the former than the latter. Scott (1955) was able to demonstrate infection in sterile, but not in unsterile soil.

In view of these differing results tests were first made by the writers on the infection of 14-day-old seedlings grown in steamed compost mixed with 1/5 (w/w) of a sand–oatmeal culture of the fungus. Thirty out of fifty seedlings showed infection in the pots within 4 weeks and the remainder succumbed within a further 6 weeks in the field. These tests were then extended to determine the longevity of the pathogen in field soil (sterile and unsterile) inoculated with spores or oat-grain culture and kept dry or moist. The soil was stored in the glasshouse in 140 3-in. pots. Twenty-eight of these were uninoculated and kept dry. The remaining 112 pots were inoculated, half of them with 1/40 (w/w) of an oat-grain culture of *Didymella* and half by adding 1 ml. per pot of a spore suspension of the fungus containing 500,000 spores per ml. Half of each group of inoculated pots were kept dry and half were watered to field capacity each week. The experiment was continued for 12 months. After 6 months, 14-day-old tomato seedlings were planted in half of the pots receiving each treatment (seven plants per treatment).

The plants remained in the pots for 4 weeks and were then planted in the field, the numbers of plants eventually contracting the disease being noted. After a further 6 months, seedlings were planted in the remaining seven pots receiving each treatment and these plants were grown on in the glasshouse. The numbers of infected plants are shown in Table 5.

TABLE 5. *The persistence of the pathogen in soil*

Treatments		Soil moisture	No. of plants with stem-base lesions* (/7)	
Soil	Inoculum		6 months	12 months
Field soil	Spore suspension	Wet	2	0
	Spore suspension	Dry	6	1
	Oat culture	Wet	7	5
	Oat culture	Dry	5	5
	No inoculum	Dry	0	0
Autoclaved field soil	Spore suspension	Wet	3	0
	Spore suspension	Dry	3	1
	Oat culture	Wet	5	6
	Oat culture	Dry	3	3
	No inoculum	Dry	0	0

* *Note.* Plants with lesions higher up the stem were not included.

These results show that the fungus persisted best when added to the soil on oat grains, which presumably served as basic food material. Even when added in the form of spore suspension, however, and thus not provided with such a food base the fungus could persist in dry soil for as long as 12 months and in moist soil (both

initially sterile and unsterile) for 6 months. This last result suggests that, in the soil used, the fungus was not subjected to active suppression by antagonistic organisms.

Bamboo canes

The importance of bamboo-cane supports as a potential source of the pathogen has been noted by several workers, who have also investigated means of disinfection. The prevailing recommendation on the treatment of canes was to immerse them completely in 5% formalin for 48 hr. This advice was apparently based on a treatment found successful by Hickman (1946), but many growers found it expensive and difficult to accomplish when many canes had to be treated. The writers accordingly tested various other ways of treating the canes with formalin and in trials made during the years 1953 to 1955 found that a modification of a method originally suggested by Kordes (1933) and Small (1937) was effective and practicable. It is now suggested that the canes should be immersed in 5% formalin for 15 min., and then wrapped for a week or more in sacks or other material. This method enables numerous canes to be treated in a relatively short time.

To determine whether it was necessary to disinfect the whole of the cane a test was made in which two canes (which had supported infected plants in the previous year) were cut into 4 in. lengths and the twenty-four portions thus obtained were tied to the stems of tomato plants. *Didymella* lesions developed on four of the plants where the portions of cane were attached. These portions were found to have been taken from widely separated parts of the canes, ranging from below soil level to the top.

Many canes taken from near infected plants have been examined for the presence of *D. lycopersici*. It proved difficult to identify the fungus but several groups of bicellular spores, identical with those of the pathogen, were seen. On one occasion, pycnidia were found on the upper part of a cane. Cultures of *D. lycopersici* were obtained from them which were pathogenic to tomato stems and fruit. It thus appears that the fungus may grow on the cane as well as existing on it in the form of spores.

Alternative hosts

There are only few reports of the natural occurrence of *D. lycopersici* on other hosts. Small (1928) showed that isolates of the fungus from strawberry plants were pathogenic to tomato stem. Van Poeteren (1929) also recorded *D. lycopersici* on strawberry. Nicholas & Aggèry (1928) and Christoff & Christova (1939) found it on egg plant, *Solanum melongena*. A similar fungus, described as *Aschochyta hortorum* was observed on artichokes by Malençon (1936).

Liesau (1932) reported that *Atropa belladona*, *Nicanda physalodes* and *Physalis franchetti* were all susceptible to infection by the tomato pathogen.

Liesau stated that the fungus occurred naturally on black nightshade, *Solanum nigrum*, which is a common weed in tomato fields. Williams *et al.* (1953) found that unwounded plants of this species did not become attacked early in the season but they did so if infected later on. The present writers found that *S. nigrum* was highly

susceptible to artificial stem inoculation, but the fungus was never observed occurring naturally on plants growing near infected tomato crops, either at the N.V.R.S. or on nurseries.

The fungus was isolated from dead potato haulm by Small (1933). Williams *et al.* (1953) found also that only senescent potato plants could be infected. No infection developed on stems of potato plants, cv. Majestic, when inoculated by the present writers in July 1953, but an isolate from old potato haulm from Ross-on-Wye was pathogenic to tomato stem and fruit. These observations suggest that *Didymella lycopersici* does not cause an active naturally occurring disease of any other plant that might serve as an important source of infection for the tomato crop in Britain, but that both the potato and *Solanum nigrum* might provide host plants on which the fungus could survive.

Spread of the disease in the field

The disease has been shown by previous workers to be spread, at least in part, by airborne spores. Liseau (1932) infected plants by blowing spores on to them and Sheard (1946, 1947) and Phillips (1955) isolated colonies of the fungus by exposing agar plates outdoors. Jenkins (unpublished data) has also demonstrated that the disease can be spread on knives used in removing sideshoots and leaves.

On some nurseries visited by the writers there was a very sudden and considerable increase in disease during September, especially in cold wet weather. Thus in one such nursery where 4100 plants were examined on 25 August 1955 twenty-two bore stem base lesions and five had lesions higher up the stem. By 30 September 1955 these numbers had increased to 154 and 203, respectively.

Observations of the apparent spread of the disease on commercial holdings are open to the criticism that the source of infection is unknown. Although such observations were made, more reliance was placed on studies of spread from artificially infected plants. Observations were first made on the development of infection following inoculation of the plants by spraying them with spore suspension obtained from cultures of *Didymella lycopersici* grown for 4 weeks on slopes of Coon's agar in 1-pint milk bottles. The spores were washed off using 300 ml. of sterile water per bottle, and the suspension was diluted appropriately after haemocytometer counts. A preliminary experiment to determine the spore concentration required for heavy infection was made by spraying the scars formed by cutting the lower leaves from 8-week-old tomato plants in the glasshouse. Five such scars were used on each plant, three plants being sprayed with 50 ml. of each concentration of spores. The results of this test are shown in Table 6.

The lowest dose giving infection of all scars was 500,000 spores per ml. (i.e. 25×10^6 per plant) and this concentration was thus used in all subsequent experiments. In numerous field trials on fungicides the treatment usually gave heavy infection, although in 1955 it was unsuccessful—probably because of the hot dry weather.

The lesions caused by the disease usually appear at the base of the plant in the earlier part of the season, leaf-scar invasion being a later phenomenon. Tests were

therefore conducted in 1956 to determine whether spraying the plants with spores would cause such basal lesions and, if so, where they occurred. In a field experiment, 8-week-old plants were given one of four treatments: (*a*) Lowest five leaves removed and stems sprayed with spore suspension; (*b*) leaves intact—base of stem sprayed with spore suspension; (*c*) five leaves removed—no spore spray; (*d*) leaves intact—no spore spray. Seventy plants were given each treatment so that ten plants (in two random plots of five plants) could be removed for examination at intervals of 1, 2, 3, 4, 6, 8 and 10 weeks. Each plant received 75 ml. of spore suspension and was carefully shielded during the process.

After 1 week most of the wounded inoculated plants had developed blackening on the edge of the leaf-scar tissue, and after 2 weeks all the scars had become heavily infected, pycnidia and spores of the fungus being present. At the 4-week inspection the lesions had coalesced and covered the stems. By this time small lesions had also appeared on the stems at soil level, mainly around the cotyledon scars.

TABLE 6. *Infection of fresh leaf scars with spore suspension*

No. spores per ml.	No. infected leaf scars/15
0	0
5,000	0
10,000	0
50,000	8
100,000	10
500,000	15
1,000,000	15

In the unwounded inoculated plants, small, rough, brown areas were observed on the hypocotyl of several plants after 1 week. Hyphae were found in this tissue but no pycnidia were present. After 2 weeks small lesions bearing pycnidia and spores of *Didymella* had developed just below soil level and below the cotyledon scars. All ten plants had become similarly affected at the 3-week sampling and the lesions had completely girdled the stem after 4 weeks. Subsequent samples showed a progressive extension of the lesion up the stem.

None of the control plants examined after 1, 2 and 3 weeks showed any infection but at the 4-week sampling one unwounded control plant bore a small lesion near a cotyledon scar and two leaf scars were infected on one wounded control plant. At each of the 6-, 8- and 10-week samplings three unwounded plants bore lesions on the hypocotyl or just above the cotyledons, but none of the wounded plants showed any leaf-scar infection. The small numbers of control plants showing infection suggests that natural spread of the disease occurred only slowly, even though the weather in 1956 was cool and wet.

Effect of irrigation. Other studies on natural spread were made in 1955 and 1956, especially in relation to the influence of overhead irrigation. This is frequently practised on commercial holdings and field evidence suggested that it might increase the rate of spread of the disease. In 1955, a hot dry year, two plots were laid out, each containing 100 plants in five rows at a spacing of 18 in. × 3 ft. with a gap across

the centre of the rows in each plot. Two plants were inoculated on 6 July on each side of this gap in each row by spraying leaf scars with spores, so that the outward spread of disease could be assessed on each side of the gap. A Kinnel oscillating spray line was placed in the gap on one plot on 2 August and this plot was sprayed twice weekly for 10 weeks using $\frac{1}{2}$ in. water on each occasion. By the end of September, seven of the eighty uninoculated plants in this plot had developed basal lesions and one bore a lesion on the stem. None of the test plants in the unirrigated plot had become infected.

A similar trial was made in 1956 using 120 test plants per plot. The infector plants were not inoculated until the end of July and the irrigation commenced 1 week later. Fourteen plants became infected on the irrigated plot, 3 at the stem base and eleven at leaf scars. Four plants were infected on the non-irrigated plot, one with a basal lesion and three at leaf scars.

It is apparent that in both seasons overhead irrigation increased the rate of spread of disease, an effect that may have been caused by the carriage of spores in the water, or by the creation of moist conditions suitable for invasion by the pathogen.

In view of the large number of infector plants (twenty per plot in each year) the extent of spread of the disease was rather low, even on the irrigated plots.

Resistance trials

Tests by previous workers have failed to reveal any marked resistance to *Didymella* stem-rot either in cultivars of *Lycopersicon esculentum* or in other species of *Lycopersicon*. Day, Jenkins & Wilcox (1956) found that all the forty lines tested from *L. esculentum*, *L. pimpinellifolium*, *L. peruvianum*, *L. glandulosum* and *L. hirsutum* were susceptible to stem inoculation although one line of each of the last two species was resistant to inoculation via the soil.

Studies on this subject were started by the writers in 1954 using thirty-six tomato cultivars and wild species. Variable numbers of each line (up to ten) were first inoculated when 6 weeks old by dipping their roots in spore suspension. The plants were then grown in the field and any that failed to show infection were re-inoculated when 11 weeks old by the insertion of a portion of an agar culture of the fungus into a stem incision. All the cultivars of *L. esculentum* proved to be susceptible to both root and stem inoculation, but some lines of wild species appeared to possess some resistance to root or stem inoculation or to both. Tests in the two succeeding years indicated that under conditions favouring the pathogen, all except one of these lines succumbed to one or both types of infection. The exception was a line of *L. hirsutum* (D 69) collected by Dr Rick of Davis, California from Canta, Peru, and sent to us by him under the number LA 94. Tests during the 3 years 1955–7 showed that this line was apparently highly resistant or immune to root inoculation and that after stem inoculation the fungus grew only on the edge of the incision. Owing to an original scarcity of seed of this line and the fact that it set no fruit from which further seed could be obtained, these results were based on observations on eleven plants only, each grown from a separate seed. All behaved in the same way, how-

ever. Cuttings were subsequently raised for further tests of the line and in 1957 it was compared with two other lines from wild species which had previously shown some resistance and with *L. esculentum* cv. Harbinger.

Up to thirty plants of each line were grown in the glasshouse in 3 in. pots and potted on 8 May into 5 in. pots each containing 25 g. of a sand–oatmeal culture of *Didymella*. Those that were not showing stem-base lesions were planted in the field on 11 June and kept until the end of September. Ten plants of each (and ten healthy Harbinger plants) were stem-inoculated on 31 July. The results are shown in Table 7. *L. hirsutum* (D 69) was the only line to remain completely free from infection and appears to possesss very high resistance. It is being used in breeding work in an attempt to transfer its resistance to commercially acceptable types. The other two lines appear to be moderately resistant and worthy of further trial.

TABLE 7. 1957 *resistance trial*

	Soil inoculation		Stem inoculation	
Tomato species and line	No. inoculated	No. infected	No. inoculated	No. infected
L. hirsutum (D 69)	30	0	10	0
L. hirsutum var. *glabratum* (D 96)	15	0	10	9*
L. peruvianum var. *dentatum* (D 23)	30	2	10	10*
L. esculentum cv. Harbinger	25	25	10	10

* *Note.* Lesions only on dead tissue around incision.

Resistance to *Didymella* is apparently not a character of all lines of the species *L. hirsutum*. Although D 69 has been shown to possess it another line (D 82) proved to be very susceptible and, as already noted, Day *et al.* (1956) found that the lines of the variety that they tested were not highly resistant.

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DIDYMELLA STEM-ROT OF OUTDOOR TOMATOES

II. STUDIES ON CHEMICAL CONTROL

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Experiments were made during 1954–7 on the control of *Didymella* stem-rot of tomatoes, by applications of various fungicides. The toxicity of a wide range of compounds to the fungus and to tomato plants was determined in the laboratory and promising compounds were then tested in the field. There were two main test methods, in both of which the plants were infected artificially at varying periods before or after treatment with the fungicides. In one method the inoculum was applied to leaf scars and in the other to the stem bases, the fungicides being sprayed on to the same sites.

Of the compounds tested, maneb was the most successful, showing both eradicant and protectant properties at 1 and 2%. Captan (0·25 and 0·5%) was a good protectant but was less effective as an eradicant. Attempts were made to test the compounds under conditions of natural spread of the disease but this was so slight that the results were not conclusive.

As part of a study of *Didymella* stem-rot of outdoor tomatoes (Knight, 1960; Knight & Keyworth, 1960) investigations were made on means of controlling the disease in the field by the application of fungicides.

Williams, Sheard & Read (1953) studied the fungicidal control of the disease in glasshouses. The stem bases of potted plants were sprayed with the test compounds and the plants were inoculated 24 hr. later by applying a thin layer of soil containing 10% of a sand–oatmeal culture of *D. lycopersici* around the plants. Only 0·5% salicylanilide gave any appreciable control. Other compounds were tested in the laboratory as soil sterilants and 0·005% ethyl mercuric phosphate gave the best results. Subsequent trials on commercial nurseries confirmed that the application of this compound at the rate of 1 gal. of a 0·006% solution per sq.yd. gave protection against reinfection.

Ethyl mercuric phosphate was used for some time on a fairly wide scale in commercial glasshouses but because of its very high human toxicity and consequent danger to operators it was eventually decided that it was unsuitable for this purpose. It was also occasionally used in the field, but here there was the added danger of contamination of the lower trusses of fruit.

In the present work an attempt was made to select relatively non-poisonous substances for use in the field. As the fungus attacks the stems, particularly at the base or around wounds, and rarely attacks the leaves, it was decided not to spray complete plants but only their stems, in the hope that the greater resistance of stems

* Including part of a thesis by this author approved for the Degree of Doctor of Philosophy of the University of London.

to phototoxic effects would enable fairly high concentrations of the compounds to be used.

Compounds were first tested for their fungitoxicity in the laboratory and for their phytotoxicity towards young tomato plants in pots. Promising compounds were then subjected to a leaf-scar infection test on the stems of mature plants in the field and later were tested as stem-base sprays.

Laboratory screening tests

It was decided that the selection of compounds on the basis of their ability to kill the spores of the pathogen would not give an adequate indication of their toxicity toward other organs of the fungus. In order to eradicate the fungus in soil near the bases of plants, or after it had become established on the plant, it was considered that the fungicide must also be capable of killing hyphae and pycnidia. A test method was therefore used in which discs of agar, bearing hyphae, pycnidia and spores of the fungus, were immersed in solutions or suspensions of the test compounds. The fungus was grown for 9 days on modified Coon's agar (maltose 7 g., KNO_3 2 g., KH_2PO_4 2·7 g., $MgSO_4$ 1·2 g., agar 20 g., water 1 l.) and discs 1 cm. diameter and approximately 3 mm. thick were cut from the agar. Six discs were gently agitated in 10 ml. of each test solution for 30 min., rinsed for 1 min. in sterile water, plated on to Coon's agar and observed for 14 days.

Fifty-seven compounds including various mercurial preparations, dithiocarbamates and antibiotics were tested in this way. Almost all the mercurial compounds killed the fungus at concentrations of less than 0·005%. They were not used in most of the field tests because of their human toxicity, but they were subsequently used as seed steeps for controlling seed-borne infection (Knight & Keyworth, 1960).

The other group of compounds showing the most promise was the metallic salts of ethylene bis-dithiocarbamate. Nabam (disodium ethylene bis-dithiocarbamate) was fungicidal at 0·02%, as was also the manganese salt (maneb) made by the addition of manganese sulphate to nabam. A similar laboratory preparation of the zinc salt (zineb) was fungicidal at 0·05% and a commercial formulation of maneb at 0·07%. A commercial formulation of zineb, however, was non-fungicidal at 6·5%. In subsequent studies of these compounds in the field, both laboratory preparations and commercial formulations were tested, the former being denoted herein by the suffix (L.).

Phytotoxicity tests

In 1954, potted plants from 7 to 13 weeks old were sprayed completely in the glasshouse with nabam, maneb (L) and zineb (L) at concentrations from 0·1 to 0·6% and quantities per plant of from 50 to 125 ml. depending on the age of the plant. Whole plants were sprayed to assess the damage likely to be caused by drift when spraying stems. All the sprays caused severe damage on 7-week-old plants but maneb (L) and zineb (L) caused no or only slight damage on 13-week plants.

Nabam damaged 13-week plants at concentrations above 0·02%. It also caused damage to 17-week-old plants in a field trial when used at a concentration of 0·3% and its use was discontinued.

In 1955 maneb (L) and zineb (L) gave no damage on 12- and 15-week-old plants in the field at concentrations up to 0·6% but caused necrotic spots on the leaves of 10-week plants.

In 1956, commercial formulations of maneb and zineb were tested on 12- and 15-week-old plants in the field as stem and leaf sprays and also as applications to the stem base. Maneb at concentrations of 0·7 and 2·1% and zineb at 0·65 and 1·95% caused no damage.

Fungicide trials in the field

These trials were made during the 4 years 1954–7 but in one of them, 1955, the weather was so hot and dry that most of the inoculations failed and the experiments were unsuccessful.

There were two main types of trial, in both of which the plants were infected artificially by applying spore suspensions. In the first type the spores and fungicides were sprayed on to leaf scars and in the second type on to the stem bases. In all trials the eradicant effects of the fungicides were assessed by applying them at various intervals after inoculation of the plants and the protectant effects were assessed by inoculating the plants at various intervals after the application of the fungicides.

The spores with which the plants were infected were obtained from 4-week-old cultures on Coon's agar of a highly pathogenic strain sent by Dr D. H. Phillips of Jersey. The suspension was made up to a concentration of 500,000 spores per ml. which had previously been found to give heavy infection. To ensure good coverage of the stems and leaf scars the spores and fungicides were applied from sprayers fitted with two opposing nozzles.

In other experiments the protectant effects of the fungicides against natural infection were determined by interspersing infected plants among the test plants. These experiments usually showed little spread of disease.

Leaf-scar tests

These tests were regarded as preliminary and were devised to enable a number of experiments to be made on relatively few plants by using each leaf scar as a test site. From five to twelve (usually seven) leaves were cut from mature plants in the field and the fresh scars were immediately sprayed with spores or fungicides, followed later by fungicide or spores, respectively. The subsequent growth of the lesion on and around the scar was graded on a scale from 0 to 5. It was found difficult to differentiate slight infection of the leaf scar (Grade 1) indicated by brown dots, from the similar dots caused by saprophytic fungi (e.g. *Stemphyllium ilicis*) and this grade was omitted from the assessment. Grades 2–5 involved increasing invasion of the stem tissue around the scar and these stages of infection were easily identified.

The same number of leaf scars was used for each treatment and the grades for each scar were summed and expressed as a percentage of the maximum possible grade (5 per scar).

1954 *trials*

In this year, only laboratory preparations of maneb and zineb were used. They were prepared as follows: 2/5 of the water required for the final spray was placed in a glass container, and the appropriate quantity of nabam was added. Manganese sulphate or zinc sulphate was then added at the rate of 1/5 (w/w) of the nabam. Finally, the remainder of the water was added and the mixture was well shaken.

Note. Figures quoted for concentrations of maneb (L) and zineb (L) refer to the concentration of nabam in the preparations.

Twelve leaves were cut off each plant and five plants were used for each concentration of fungicide. Approximately 100 ml. of spore suspension and 250 ml. of fungicide was applied per plant (in one dose) and the final disease assessment was made 6 weeks after inoculation.

The results of this trial showed that 0·6% maneb (L) and zineb (L) considerably reduced infection if applied up to 7 days after inoculation but there was no such eradicant effect if the fungicidal application was delayed for 14 days. Both compounds also gave protection against infection if the plants were inoculated up to 7 days after the fungicides had been applied.

1956 *trials*

The experiments were extended in 1956 to include proprietary formulations of maneb and zineb and also certain other fungicides.

The plants were 16 weeks old when treated and seven leaves were cut from the middle part of each stem: 75 ml. of spore suspension and 100 ml. of fungicide were used per plant. The use of a constant-pressure sprayer enabled the quantities of fungicide to be judged accurately on a timing basis. Each treatment was applied to fifteen plants in three five-plant plots arranged in randomised blocks and final records were made 8 weeks after inoculation.

The results of the eradicant and protectant trials with maneb and zineb are given in Table 1. It was apparent from these results that maneb (2·1%) and maneb (L) (0·6%) had similar eradicant properties, giving considerable disease reduction if applied up to 5 days after inoculation. Zineb and zineb (L) were slightly less effective and the eradicant effect of all the compounds was greatly reduced if their application was delayed for 7 days. Both maneb and zineb were good protectants.

In another series of leaf-scar trials of similar design to those above the manganese and zinc dimethyl dithiocarbamate derivatives were tested together with captan and thiram. Treatments with zineb (L) were included for comparison. The results are given in Table 2.

It is apparent from these results that, at the concentrations used, the dimethyl compounds of manganese and zinc were relatively ineffective either as eradicants or protectants. Both thiram and captan gave some measure of eradication if applied

3 days after inoculation but neither was as effective as zineb (L). It is clear, however, that both, and especially captan, had a considerable protectant effect. The relatively small amount of disease on the plants inoculated 7 days after fungicidal application in the protectant trial is thought to be related to the weather at the time of inoculation coupled with the decrease in susceptibility of the leaf scars of ageing.

TABLE 1. 1956 *leaf-scar trials of maneb and zineb*

		Disease indices (% max.)					
		Eradicant trial (compounds applied after)			Protectant trial (spores applied after)		
Compound	Concn. (%)	3 days	5 days	7 days	3 days	5 days	7 days
Maneb (L)	0·6	6·0	13·0	37·0	15·0	9·5	10·5
Maneb	0·7	19·0	32·0	37·0	11·0	19·5	27·5
Maneb	2·1	4·5	11·0	29·5	2·0	1·5	10·5
Zineb (L)	0·6	14·0	13·5	38·0	9·5	21·5	9·5
Zineb	0·65	37·0	—	35·0	2·5	9·0	9·5
Zineb	1·95	12·0	33·5	23·0	6·0	7·0	4·5
Nil (inoculated)	—	57·0	54·5	64·0	66·5	88·5	46·5
Nil (uninoculated)	—	2·5	0	1·5	0·5	2·0	0·5

TABLE 2. 1956 *leaf-scar trials of other compounds*

		Disease indices (% max.)			
		Eradicant trial (compounds applied after)		Protectant trial (spores applied after)	
Compound	Concn. (%)	3 days	7 days	3 days	7 days
Manganese dimethyl dithiocarbamate	0·43	53·0	65·0	40·0	16·0
Ziram	0·76	73·5	80·0	20·5	3·6
Thiram	0·8	23·0	63·5	15·5	8·0
Captan	0·5	24·5	61·0	2·5	1·5
Zineb (L)	0·6	9·5	35·0	12·0	10·5
Nil (inoculated)	—	83·0	90·0	51·5	30·5
Nil (uninoculated)	—	17·5		3·0	

1957 *trials*

Further leaf-scar trials were made during 1957, using commercial formulations of maneb and zineb and comparing these with captan and with Karathane W.D. (dinitrocaprylphenyl crotonate). In the eradicant test the fungicides were applied 5 days after inoculation and in the protectant test the leaf scars were inoculated 5 days after the fungicides had been applied. There were fifteen plants per treatment in three five-plant plots arranged in randomized blocks and seven leaf scars were used on each plant.

The results after 8 weeks are given in Table 3.

The results confirm the eradicant effect of maneb and zineb shown in previous

trials and also suggest that captan may have some eradicant properties, at least at a concentration of 0·5%. Karathane W.D. had little eradicant effect but all the materials gave a high degree of protection.

TABLE 3. 1957 *leaf-scar tests*

Compound	Concn. (%)	Disease indices (% max.) Eradicant test	Protectant test
Maneb	1	12·5	2·5
	2	10·0	2·0
Zineb	1	17·0	2·5
	2	11·0	1·0
Captan	0·1	20·0	3·5
	0·25	26·5	3·0
	0·5	10·5	3·0
Karathane W.D.	0·5	28·0	3·5
	1	31·5	2·0
Nil (inoculated)	—	51·0	34·5
Nil (uninoculated)	—	2·5	0

Stem-base tests

The primary infection site of the pathogen is at the base of the stem and studies showed that such infections could be obtained artificially by placing solid inocula around the stems or by spraying the base of the stem with spores. Experiments were therefore made in 1956 and 1957, using the materials found most effective in the leaf-scar tests, to determine whether spraying the compounds on the stem base and surrounding soil would suppress the basal infections.

1956 *trial*

This trial was largely exploratory and was designed to test whether infection could be controlled by one application of the compounds at various times after or before inoculation or whether subsequent additional treatments were necessary. The plants were inoculated by placing 100 g. of a 1/4 (w/w) mixture of a sand–oatmeal culture of the fungus and autoclaved soil about $\frac{1}{2}$ in. on one side of the stem and covering it with soil. There were fifteen plants per treatment in three five-plant plots arranged in randomized blocks. In the eradicant test, the fungicides were applied at the rate of 150 ml. per plant either once, 7 days after inoculation or three times, 7, 21 and 42 days after inoculation. In the protectant tests the plants were inoculated 3 days after the application of the fungicides, one set receiving one dose of fungicide only and another set receiving two additional fungicidal treatments 21 and 42 days later. The numbers of plants which had developed stem-base lesions during a period of 8 weeks after inoculation are shown in Table 4.

There were several apparent inconsistencies in the results from this trial and it was clear that the method of inoculation did not give a very high degree of infection. There was a clear indication, however, that in the eradicant test the application of further sprays, especially of maneb, caused a greater reduction of infection than the

use of one spray only. In the protectant trial the use of additional sprays of the highest concentration of maneb completely eliminated the disease, but the single-spray treatments were less effective than when the same compounds were used as eradicants. This may have been due to a loss of effect during the 3 days that the compounds were in the soil before the inoculum was applied.

TABLE 4. 1956 *stem-base tests*

		No. infected plants/15			
		Eradicant test		Protectant test	
Compound	Concn. (%)	One spray	Three sprays	One spray	Three sprays
Maneb (L)	0·6	7	1	12	4
Maneb	0·7	4	2	10	7
Maneb	2·1	5	1	10	0
Zineb (L)	0·6	3	1	12	5
Zineb	0·65	5	8	13	12
Zineb	1·95	4	2	8	9
Nil (inoculated)	—	11	—	10	—
Nil (uninoculated)	—	1	—	1	—

1957 *trials*

In this season the stem-base tests were extended. Two methods of inoculation were used, viz. solid inoculum in the soil, and sprays of the stem base with spore suspension. Captan and thiram were included and some tests were made in which the plants were treated in the pots before planting out.

(1) *Stem-base test of maneb, zineb and captan.* Commercial formulations of the three compounds were used and their effect was compared with that of ethyl mercuric phosphate. There were two methods of inoculation: the use of sand–oatmeal culture as in 1956 and the application of 55 ml. per plant of a spore suspension (500,000 spores per ml.). There were twenty plants per treatment in four randomized blocks and the compounds were applied at the rate of 150 ml. per plant either 5 days before or 5 days after inoculation (eradicant and protectant tests, respectively) and on two subsequent occasions (after 21 and 42 days).

The numbers of plants which developed stem-base lesions during a period of 12 weeks after inoculation are shown in Table 5.

It appeared from the results of this trial that the spore inoculation provided a severer test of the potentialities of the fungicides than the use of solid inoculum. Both methods, however, showed that maneb was successful both as an eradicant and as a protectant. Ethyl mercuric phosphate was a good eradicant but not a good protectant, whereas the converse applied in the case of captan. Zineb was not very successful in either capacity.

(2) *Protectant test of captan and thiram.* This was a comparison of the effects of the compounds applied once before or after planting with those when they were applied after planting and also subsequently. There were twenty plants per treatment and they were 6 weeks old when put into the field (i.e. somewhat younger than usual). In one series of treatments the fungicides were applied to the potted plants

at the rate of 25 ml. per plant 3 days before planting. In another series the plants were treated 3 days after planting, using 50 ml. of fungicide per plant, and in a third series this treatment was repeated four more times at fortnightly intervals. The spore inoculum (40 ml. per plant) was applied 6 days after planting (i.e 3 days after the first field treatment and 9 days after the pot treatment). Recording of infected plants was continued for 16 weeks and in Table 6 are shown the numbers of plants which had developed stem-base lesions after 10, 14 and 16 weeks.

TABLE 5. *Stem-base test of maneb, zineb, captan and ethyl mercuric phosphate*

		No. infected plants/20			
		Eradicant trial		Protectant trial	
Compound	Concn. (%)	Spore inoculum	Culture inoculum	Spore inoculum	Culture inoculum
Maneb	1	3	0	4	0
	2	3	1	0	0
Zineb	1	10	5	11	7
	2	8	5	9	5
Captan	0·25	10	5	3	3
	0·5	6	4	2	3
E.M.P.	0·006	3	1	7	6
Nil (inoculated)	18	18	19	20	17
Nil (uninoculated)		3	0	2	3

TABLE 6. *Test of captan and thiram applied before and after planting*

			No. infected plants/20		
Compound	Concn. (%)	Time of treatment*	10 wk.	14 wk.	16 wk.
Captan	0·25	B.P.	12	18	19
		A.P.	0	4	5
		A.P.+4	0	0	1
	0·5	B.P.	3	9	15
		A.P.	0	2	5
		A.P.+4	0	0	1
Thiram	0·25	B.P.	19	19	19
		A.P.	8	10	17
		A.P.+4	1	3	7
	0·5	B.P.	16	19	20
		A.P.	6	11	16
		A.P.+4	0	1	4
Nil (inoculated)		—	20	20	20
Nil (uninoculated)		—	1	3	3

* *Note.* B.P., before planting; A.P., after planting; A.P.+4, after planting plus 4 additional treatments.

Thiram was not as effective as captan but five applications of the compound caused a marked reduction in disease. Captan gave a very striking control, especially when applied at five fortnightly intervals, but it was also very effective when applied once after planting. The fact that considerable protection was given

for a period of 10 weeks by a single application to the potted plants before they were put into the field is also noteworthy and indicates a very high degree of suppression of subsequent infection.

Trials of fungicides against natural infection

It was considered that the field testing of fungicides, by methods involving artificial infection of the plants, was open to the criticism that the conditions of infection might be dissimilar from those occurring when the plants became infected from nearby plants. Numerous attempts were made, therefore, to test the effectiveness of the fungicides against natural infection. These usually took the form of interspersing artificially infected plants among uninoculated treated plants or treating selected healthy plants in heavily infected commercial plantations. In general, insufficient infection of the test plants was obtained to enable the effectiveness of the fungicides to be assessed, but some trials gave results of value.

In 1954, 150 apparently healthy plants in five scattered groups each containing thirty plants were selected in a heavily infected nursery and ten leaves were removed from every plant. The leaf scars on ten plants in each group were sprayed with maneb (L), those on ten other plants with zineb (L) and those on the remaining ten plants were untreated. The fungicides were re-applied 3 weeks later. The results are shown in Table 7.

Table 7. *Leaf-scar trial on a commercial nursery*

Compound	Concn. (%)	Disease index (% max.) After 4 wk.	Disease index (% max.) After 6 wk.
Maneb (L)	0·6	6·5	21·0
Zineb (L)	0·6	5·0	23·5
Nil	—	28·5	60·0

Both treatments gave a marked reduction in disease. A similar trial was made in 1956 using zineb (L) at 0·6 and 0·2% but the amount of disease on the control plants was too low to enable the effects of the compound to be judged adequately. At the higher concentration, however, the disease index was only 0·5%, compared with 8·0% on the controls.

On the same nursery fifty plants were selected at random and 0·6% zineb (L) was applied at seven fortnightly intervals to the bases of twenty-five of them at the rate of 150 ml. of the compound on each occasion. None of the treated plants showed any infection whereas seven of the untreated plants developed stem-base lesions.

A trial was made in 1956 in which the bases of plants in groups surrounding artificially infected plants were sprayed with various compounds. Four out of eighteen untreated plants contracted the disease within 8 weeks whereas none of eighteen plants sprayed with 2·1% maneb showed any infection.

In 1957 a more extensive trial was made in which the plants were grown at a spacing of $1\frac{1}{2} \times 5$ ft. and (except in healthy control plots) one-third of them were infected by the insertion of a culture of the fungus into an incision near the base of

the stem. The stem bases of the remaining plants were sprayed with various compounds (150 ml. per plant) either six times at fortnightly intervals or four times at 3-week intervals, there being twenty-four plants per treatment. The first spray was given on the day before the infector plants were inoculated.

The numbers of test plants showing stem-base lesions during a period of 16 weeks after the infectors were inoculated is shown in Table 8.

TABLE 8. *Test of fungicides against natural infection*

Compound	Concn. (%)	No. of infected plants/24	
		6 treatments	4 treatments
Maneb	1	0	0
	2	0	0
Zineb	1	2	2
	2	4	0
Captan	0·25	1	2
	0·5	0	1
Ethylmercuric phosphate	0·006	1	8
Nil (infectors inoculated)	—	3	
Nil (infectors uninoculated)	—	6	

Only nine of the forty-eight control plants became infected, indicating that relatively little spread of the disease occurred. Even so, the fact that none of the ninety-six plants treated with maneb (at 1 and 2%) and only one of the forty-eight plants treated with 0·5% captan developed the disease suggests that both fungicides were protecting the plants against infection carried from nearby infectors. The results of all the natural-infection tests are in agreement with those obtained by the methods involving artificial infection and suggest that the latter did give a reliable indication of the potentialities of the fungicides.

DISCUSSION

The experiments on the control of *Didymella* stem-rot of outdoor tomatoes by means of fungicidal applications show that certain compounds gave a considerable reduction in disease and their use merits further trial. Relatively high concentrations of the dithiocarbamates were necessary to ensure adequate control and this fact may make the use of these compounds uneconomic on the outdoor crop. On the other hand, the localized application reduces the quantities required and would offset this difficulty to some extent. On a cost basis captan would appear to be the most useful material of those tested, and in this connexion the success of the single applications immediately after planting is worthy of note.

No tests have been made by the writers on the control of the disease in glasshouses and the compounds would obviously require further trial before they could be recommended for this very different type of culture. A few reports have been

received on the use of these compounds in commercial glasshouses which suggest that captan may be effective under these conditions. Maneb, on the other hand, has been reported to have caused severe phytotoxicity in some glasshouses. This conflicts with the results on the outdoor crop and the cause is at present unknown, but it is obvious that the material should be used with caution and only on an experimental basis.

Thanks are due to those growers and officers of the N.A.A.S. who have collaborated in the commercial trials and to The Murphy Chemical Co. Ltd., Pan Britannica Industries Ltd., and Plant Protection Ltd., who supplied certain of the commercial formulations used in the experiments.

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ANTIFUNGAL ACTION OF STREPTOMYCIN-COPPER CHELATE AGAINST *PHYTOPHTHORA INFESTANS* ON TOMATO (*LYCOPERSICON ESCULENTUM*)

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(With 1 Text-figure)

The antifungal protection afforded by streptomycin-copper chelate *in vivo* in greenhouse tests against *Phytophthora infestans* on tomato was found to be of the order of six times greater than that of streptomycin sulphate. The copper chelate also showed much greater resistance to simulated rainfall. Similar tests showed that streptomycin-copper chelate (in terms of streptomycin content) was from 2½ to 5 times more effective as an antifungal prophylactic spray than zinc ethylene-bis(dithiocarbamate) (zineb), and some fifty times more active than copper oxychloride formulated as a commercial 50% wettable powder. These three materials showed roughly similar resistance to weathering by 'rainfall' and it was assumed therefore that 250 i.u./ml. streptomycin as streptomycin-copper chelate might be expected to give the same degree of protection against *Phytophthora infestans* in the field as would the usual field rates of usage of zineb and copper oxychloride.

Introduction

The antibacterial antibiotic streptomycin has no inhibitory effect on fungi *in vitro*, but small quantities absorbed by roots of tomato and potato plants have exhibited substantial antifungal activity in the foliage (Muller, Mackay & Friend, 1954). Infection was not prevented, but spread of the pathogen within the host was greatly restricted. The antifungal protection was attributed to modification of the host-plant's physiology in the presence of streptomycin.

Streptomycin is also effective as a foliage spray in small-scale tests, good protection having been obtained against *Peronospora tabacina* on tobacco (Grosso, 1954; Anderson, 1956), *Pseudoperonospora cubensis* on cucumber (Coe, 1955), *Phytophthora phaseoli* on lima bean (Zaumeyer & Wester, 1956) and *P. infestans* on tomato (Zaumeyer & Doolittle, 1956).

This antifungal action of streptomycin *in vivo* can be increased by admixture with water-insoluble copper fungicides (Chas. Pfizer & Co. Inc., 1958), and it has been suggested that the mechanism behind streptomycin-induced resistance to phycomycete fungal pathogens may lie in the increased polyphenolase (a copper enzyme) activity of the host plant (Vörös, Király & Farkas, 1957).

Therefore when a method for preparation of streptomycin-copper chelate was described (Foye, Lange, Swintosky, Chamberlain & Guarini, 1955), we were interested to determine if this new complex would show greater antibiotic activity than simple mixtures of streptomycin with copper or the ingredients separately.

Preliminary greenhouse tests established the fact that streptomycin-copper chelate gave both direct and systemic protection against *Pseudomonas medicaginis* var. *phaseolicola* (halo blight of dwarf bean) at least equal to that of streptomycin sulphate or mixtures of streptomycin sulphate with copper oxychloride. The antibacterial tests were followed by an evaluation of the antifungal activity of streptomycin-copper chelate against *Phytophthora infestans* on tomato and these observations are now reported.

Experimental method

A greenhouse technique for evaluation of fungicides against *P. infestans* on tomato was developed from a method described in 1943 (McCallan & Wellman, 1943).

Stonor's M.P. tomato plants, grown under fluorescent strip lights for about 4 weeks, were used when the sixth compound leaf was clearly visible. The pathogen was a virulent strain of *P. infestans* (Mont.) de Bary, provided by Dr Mary Noble of the Department of Agriculture for Scotland, and maintained thereafter by subculture every 14 days on chick-pea medium (Keay, 1953).

In each experiment, forty-nine plants were used in a 7×7 Latin square. The suspensions or solutions without wetter were applied to the dorsal surfaces of the second, third and fourth leaves above the cotyledons, by means of a B.E.N. model R.F.4. spray-gun at a constant pressure of 10 lb./sq.in., so as to wet the leaflets completely but without 'run-off'.

Inoculum was prepared from a 14-day culture of *P. infestans* on chick-pea medium by immersing a test-tube containing a suspension of 20,000 sporangia/ml. for 6 hr. in running tap water (about 10° C.) to permit maximum release of zoospores. 24 hr. after treatment, this suspension of zoospores was applied with a B.E.N. model S no. 1 spray-gun at a pressure of 10 lb./sq.in. to the dorsal surfaces only of the three treated leaves on each plant, with no more zoospore suspension than was necessary to give a uniform cover of small but discrete droplets of fluid to the leaf surfaces.

When inoculation was completed, the plants were transferred to a polythene humidity chamber cooled by an external spray of cold water, where the atmosphere was maintained as nearly as possible at saturation point, until discrete lesions had developed 65 hr. later. Although the incubation time was not very critical, it was observed that excessive incubation or delay in lesion count resulted in an aggressive manifestation of the disease where lesions fused, leaflets wilted and epinasty developed.

It was found convenient to view the lesions from the ventral surfaces of the leaves against a light background. The aggregate of lesions on the three inoculated leaves of each plant was taken as a measure of the severity of disease. The mean number of lesions on seven treated plants compared with the mean number on corresponding leaves of control plants gave an estimate of the antifungal protection afforded by the treatment.

Method of statistical analysis

Inspection of the results showed that the error variation was not homogeneous: it was dependent on the mean number of lesions, which in turn was dependent on the treatment. An approximate calculation demonstrated that the standard error was nearly always proportional to the mean: a logarithmic transformation was therefore applied to render the variance homogeneous.

Materials

Pharmaceutical-grade streptomycin sulphate (bio-assay against *B. subtilis* 740 i.u./mg.), copper oxychloride (50% wettable powder) and a formulation of 50% zinc ethylene-bis(dithiocarbamate) (zineb) were used for comparison.

The streptomycin-copper chelate was prepared for us by our colleague Mr A. R. Lockwood; it contained 13% (w/w) of copper and gave a bio-assay against *B. subtilis* of 475 i.u./mg.

Streptomycin sulphate, being readily water-soluble, was used in solution, but streptomycin-copper chelate and the other materials were used as suspensions owing to their limited solubility in water.

RESULTS

We report five experiments in which the antifungal action of streptomycin-copper chelate was compared with those of streptomycin-sulphate, copper oxychloride, zineb [zinc ethylene-bis(dithiocarbamate)] and mixtures of streptomycin sulphate with copper oxychloride; and two experiments in which the effect of simulated rainfall on the antifungal activity of these materials was observed. The results are given in Tables 1–7 and the corresponding analyses of variance are summarized in Table 8.

Experiment 1. *Comparison of streptomycin-copper chelate with streptomycin sulphate*

Streptomycin-copper chelate and streptomycin sulphate were compared on the basis of equal weights at two concentrations. When the appropriate corrections for potencies of the two samples were made, the experiment compared 74 and 37 i.u./ml. streptomycin as streptomycin sulphate against 47·5 and 23·5 i.u./ml. streptomycin as streptomycin-copper chelate.

The results (Table 1) show that streptomycin-copper chelate gave markedly less incidence of disease than streptomycin sulphate, the mean log. lesion number at the lower level of the former being significantly less than at the higher level of streptomycin sulphate, although both materials had significant antifungal action, which became more marked with increase in concentration.

Experiment 2. *Comparison between streptomycin-copper chelate and zineb*

Streptomycin copper-chelate was compared on an equal weight basis with zinc ethylene-bis(dithiocarbamate). Streptomycin-copper chelate was used at 100, 33·3 and 11 μg./ml., equivalent to 47·5, 15·8 and 5·2 i.u./ml. streptomycin, respectively, and zineb at levels giving 100, 33·3 and 11 μg./ml. of active ingredient.

Table 2 shows that, whereas zineb produced a significant reduction in disease only at the highest level (100 μg./ml. active ingredient), streptomycin-copper chelate showed significantly less incidence of disease at all three levels.

There was a suggestion, however, that the dose-response curve for zineb may be steeper than that for streptomycin-copper chelate, as the antifungal action of zineb rapidly increased once the critical level had been reached, so that at 100 μg./ml. active ingredient zineb gave control equivalent to that obtained from an equal concentration of streptomycin-copper chelate (47·5 i.u./ml. streptomycin equivalent).

TABLES 1–7. *Comparisons of antifungal action of streptomycin-copper chelate and other materials*

TABLE 1. *Comparison of streptomycin-copper chelate with streptomycin sulphate*

Concn. of formulated material (μg./ml.)	Concn. of active ingredient (i.u./ml. strep.)	Material	Total lesion count	% reduction on control. $\frac{C-T}{C} \times 100$	Geom. mean lesion count	Log. geom. mean lesion count
100	74	Strep. sulphate	1126	60	139	2·144*
50	37	Strep. sulphate	2034	28	256	2·409*
100	47·5	Strep.-Cu chelate	344	88	43	1·634*
50	23·7	Strep.-Cu chelate	518	82	72	1·858*
		(Other treatments)†	2690	5	374	2·573
		(Other treatments)†	2622	7	361	2·557
		Control	2822	—	376	2·575
				S.E. of difference		0·07

TABLE 2. *Comparison between streptomycin-copper chelate and zineb*

100	47·5 i.u./ml.	Strep.-Cu. chelate	527	83	71	1·852*
33·3	15·6 i.u./ml.		992	68	121	2·083*
11·1	5·2 i.u./ml.		1597	48	196	2·293*
200	100 μg./ml.	Zineb	644	79	63	1·796*
66·6	33·3 μg./ml.		1953	37	240	2·380
22·2	11·1 μg./ml.		2309	25	313	2·496
		Control	3071	—	377	2·576
				S.E. of difference		0·10

TABLE 3. *Evaluation of streptomycin-copper chelate against a mixture of streptomycin sulphate with copper oxychloride*

Concn. of copper (μg./ml.)	Concn. of streptomycin (i.u./ml.)					
13	47·5	Strep.-Cu chelate	385	90	51	1·710*
4·3	15·8		853	80	106	2·026*
1·4	5·2		2030	50	264	2·421*
13	47·5	Strep. sulphate + Cu oxychloride	1293	68	161	2·206*
4·3	15·8		2592	36	310	2·491*
1·4	5·2		3380	17	394	2·595
		Control	4068	—	500	2·699
				S.E. of difference		0·07

TABLE 4. *Comparison of streptomycin-copper chelate with mixtures containing various proportions of copper to streptomycin*

Concn. of copper	Concn. of streptomycin (i.u./ml.)	Material	Total lesion count	% reduction on control. $\frac{C-T \times 100}{C}$	Geom. mean lesion count	Log. geom. mean lesion count
13	47·5	Strep.-Cu chelate	1158	82	152	2·182*
Nil	47·5	Strep. sulphate	3018	52	374	2·573*
13	47·5	Strep. sulphate+ Cu oxychloride	3060	52	354	2·549*
47·5	47·5		2482	61	295	2·470*
237·5	47·5		1773	73	208	2·319*
1187·5	47·5		854	86	110	2·041*
		Control	6334	—	820	2·914
				s.e. of difference		0·07

TABLE 5. *Dose responses of streptomycin-copper chelate and of zineb*

Concn. of formulated material (μg./ml.)	Concn. of active ingredient					
200	95 i.u./ml.	Strep.-Cu chelate	284	97	33	1·523*
100	47·5 i.u./ml.		512	93	60	1·776*
50	23·7 i.u./ml.		1173	84	145	2·160*
25	11·8 i.u./ml.		1564	78	199	2·298*
12·5	5·9 i.u./ml.		2771	62	344	2·537*
500	250 μg./ml.	Zineb	170	98	15	1·166*
		Control	7396	—	1019	3·008
				s.e. of difference		0·13

TABLE 6. *The effect of simulated 'rainfall' on the antifungal protection afforded by streptomycin-copper chelate, streptomycin sulphate or copper oxychloride*

30	14·2 i.u./ml.‡	Strep.-Cu chelate	1362	63	156	2·193*
30	14·2 i.u./ml.§	Strep.-Cu chelate	2156	41	280	2·447
150	111 i.u./ml.‡	Strep. sulphate	1140	69	79	1·896*
150	111 i.u./ml.§	Strep. sulphate	2844	23	329	2·517
3000	1500 μg./ml.‡	Cu oxychloride	658	82	83	1·917*
3000	1500 μg./ml.§	Cu oxychloride	1516	59	179	2·252*
		Control	3677		482	2·683
				s.e. of difference		0·12

TABLE 7. *The effect of simulated 'rainfall' on the protectant fungicidal values of streptomycin-copper chelate, copper oxychloride and zineb*

30	14·2 i.u./ml.‡	Strep.-Cu chelate	1013	71	129	2·112*
30	14·2 i.u./ml.§	Strep.-Cu chelate	1508	57	210	2·322*
2000	1000 μg./ml.‡	Cu oxychloride w.p.	687	81	90	1·955*
2000	1000 μg./ml.§	Cu oxychloride w.p.	1028	71	134	2·128*
150	75 μg./ml.‡	Zineb	1257	64	123	2·089*
150	75 μg./ml.§	Zineb	1746	50	242	2·384*
		Control	3493	—	483	2·684
				s.e. of difference		0·10

* Significantly different from control at $P = 0·05$.
† Treatments not relevant to the results reported were included in the estimate of error.
‡ Treatment: 'dry'. § Treatment: 'rain'

Experiment 3. *Comparison of streptomycin-copper chelate with a mixture of streptomycin sulphate and copper oxychloride*

The streptomycin-copper chelate contained 13% (w/w) copper and it was therefore considered necessary to determine whether the chelate complex was more active than a mixture containing equivalent amounts of copper and streptomycin. Streptomycin-copper chelate suspensions equivalent to 47·5, 15·8 and 5·2 i.u./ml. streptomycin were compared with mixtures of streptomycin sulphate and copper oxychloride containing 47·5 i.u./ml. streptomycin with 13 μg./ml. copper, 15·8 i.u/ml. streptomycin with 4·3 μg./ml. copper and 5·2 i.u./ml. streptomycin with 1·43 μg./ml. copper.

TABLE 8. *Summary of analyses of variance* (*mean squares*)

Source	Degress of freedom	Experiment number 1	2	3	4	5	6	7
Rows	6	0·1378*	0·3488*	0·3468*	0·3617*	0·5063*	0·5806*	0·1384*
Columns	6	0·0253	0·0368	0·0263	0·0170	0·0730	0·0389	0·0222
Treatments	6	1·0197*	0·6618*	0·8505*	0·5782*	2·7535*	0·6234*	0·4174*
Error	30	0·0194	0·0360	0·0165	0·0160†	0·0629	0·0469	0·0349

* Significant at the 5% level of probability.
† 29 degrees of freedom for error.

Table 3 shows that streptomycin-copper chelate was at least three times more active than the mixture of streptomycin with copper, the lowest level (equivalent to 5·2 i.u./ml. streptomycin) again giving approximately 50% reduction in disease. There was also a suggestion that the dose-response curve for streptomycin-copper chelate may be steeper than that for streptomycin mixed with copper oxychloride.

Experiment 4. *Comparison of streptomycin-copper chelate with mixtures containing various proportions of copper to streptomycin*

100 μg./ml. streptomycin-copper chelate, equivalent to 47·5 i.u./ml. streptomycin, and containing 13 μg./ml. copper, was compared with an equivalent amount of streptomycin as streptomycin sulphate alone or the same amount of streptomycin mixed with increasing quantities of copper as copper oxychloride. The concentrations of copper ranged from 13 μg./ml. (equivalent to the copper contained in the streptomycin-copper chelate treatment) to 1187·5 μg./ml. (equivalent to a 25: 1 ratio of copper to streptomycin).

It may be concluded from Table 4 that there was a significant decrease in mean lesion count as the level of copper in the mixture increased, though the mixture required a 25: 1 ratio of copper to streptomycin before the control of disease surpassed that given by streptomycin-copper chelate.

Experiment 5. *Comparison of dose responses to streptomycin-copper chelate and zineb*

A dose-response curve for streptomycin-copper chelate was determined by using the material at concentrations equivalent to 95, 47·5, 23·8, 11·9, 5·9 i.u./ml. streptomycin. Zineb was included at 0·05%, equivalent to 250 μg./ml. zinc ethylene-bis(dithiocarbamate).

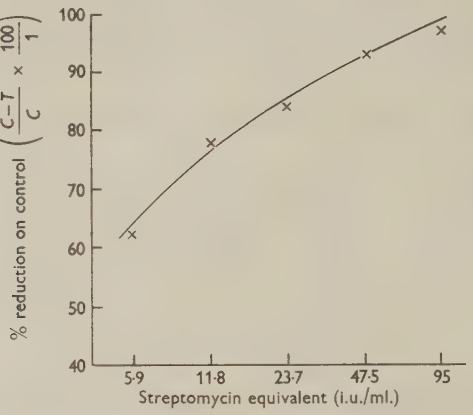

Fig. 1. Dose-response curve of streptomycin-copper chelate (Exp. 5).

The observed dose-response curve [percentage reduction of disease relative to control plotted against log. concentration (i.u./ml. streptomycin equivalent)] is given in Fig. 1, and the lesion counts after streptomycin-copper chelate or zineb treatment are shown in Table 5. All treatments gave a significant reduction in disease, and each increment in streptomycin-copper chelate concentration significantly increased disease control. It may be concluded that, under the conditions of this experiment, 95% protection would be achieved by approximately 66 i.u./ml. streptomycin in the form of streptomycin-copper chelate and that zineb would be expected to give equivalent protection at somewhat less than 250 μg./ml. active ingredient.

Experiment 6. *The effect of simulated 'rainfall' on the antifungal protection afforded by streptomycin-copper chelate, streptomycin sulphate and copper oxychloride*

To evaluate the rain-resistance of streptomycin-copper chelate the suspensions or solutions were applied and allowed to dry in the usual manner. Half the plants were maintained with dry foliage, and the remainder were placed at random on the floor of the greenhouse within an area of 6 × 6 ft. Rainfall was simulated by covering the plants with a uniform spray of fine water droplets distributed from four jets (MacPenny's mist propagation) mounted on supports 30 in. high at the corners of the square. In this manner, water was sprayed continuously at a rate equivalent to $\frac{1}{2}$ in. rainfall in about 30 min. When 'rainfall' was completed, the foliage was

allowed to dry and then both 'rain' and 'dry' plants were inoculated and subsequently incubated in the usual manner.

By this means the effect of 'rainfall' was determined on streptomycin-copper chelate, streptomycin-sulphate and copper oxychloride foliage sprays applied at concentrations known to give about 70% reduction in disease in our standard greenhouse tests. The results (Table 6) show that 'rainfall' significantly lowered the antifungal protection afforded by each substance, but that this loss of efficiency was significantly greater with streptomycin sulphate than with the other two materials, each of which was affected to about the same degree.

This experiment also demonstrates that streptomycin-copper chelate is much more effective as a prophylactic spray than copper oxychloride, which at an active ingredient concentration of 1500 μg./ml. gave only 82% reduction of disease compared with the untreated control.

Experiment 7. The effect of simulated 'rainfall' on the antifungal protection afforded by streptomycin-copper chelate, copper oxychloride or zineb

This experiment was similar to experiment 6, but the effect of 'rainfall' on streptomycin-copper chelate was compared with its action on foliage sprays of copper oxychloride and zineb, all treatments again being chosen to give about 70% reduction in lesion count in the absence of 'rainfall'. Table 7 shows that the mean lesion counts for all treatments increased after 'rainfall'. There was, however, no significant difference between the three materials in relation to loss of efficiency after 'rainfall', although the change in mean lesion counts suggested that the copper oxychloride may have suffered somewhat less than the streptomycin-copper chelate and zineb.

Again it may be seen that streptomycin-copper chelate as a prophylactic foliage spray is considerably more active than either copper oxychloride or zineb, 14·2 i.u./ml. streptomycin-copper chelate giving protection not significantly different from that given by 75 μg./ml. zineb or 1000 μg./ml. copper oxychloride active ingredients.

Discussion

These experiments show that in these greenhouse trials the antifungal protection afforded by streptomycin-copper chelate *in vivo* is about six times greater than that of streptomycin sulphate. It is possible that the difference might be accentuated under field conditions owing to the greater resistance of the chelate to rainfall, thus suggesting that streptomycin-copper chelate might be tried against those phycomycete diseases for which streptomycin salts are marginally economic, particularly when the fungus is present with a bacterial pathogen.

Zineb and copper oxychloride probably represent the best available commercial fungicides for control of *Phytophthora infestans* on tomato and potatoes, and our greenhouse trials indicate that streptomycin-copper chelate (in terms of streptomycin content) is from $2\frac{1}{2}$ to 5 times more effective as an antifungal prophylactic foliage spray than zinc ethylene-bis(dithiocarbamate) and some fifty times more

active than copper oxychloride formulated as a commercial 50% wettable powder. Since these three materials showed roughly similar resistance to weathering by 'rainfall', it might be assumed that approximately 250 i.u./ml. streptomycin as streptomycin-copper chelate would give the same degree of protection against *P. infestans* in the field as would the usual field rates of usage of zineb (0·075% of active ingredient as 50% wettable powder) and copper oxychloride (0·25% of active ingredient as 50% wettable powder). Results of such field tests are not yet available.

The improved antifungal control obtained with streptomycin-copper chelate is an induced response, for the small amount of copper held in the complex is ineffective alone, and indeed much larger amounts of copper in simple mixture with streptomycin sulphate are not as effective as the chelate complex.

Like streptomycin salts, streptomycin-copper chelate has no fungistatic action *in vitro* (Foye *et al.* 1955), and it seems therefore that it also may act by modifying the host plant's metabolism in favour of increased activity of the copper-containing enzyme, polyphenolase (Vörös *et al.* 1957), perhaps owing to less rapid dissipation of the streptomycin-copper chelate, of low water-solubility, within the host tissues.

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STUDIES ON *GLOEOSPORIUM MUSARUM* CKE. & MASSEE CAUSING STORAGE ROTS OF JAMAICAN BANANAS

I. ANTHRACNOSE AND ITS CHEMICAL CONTROL

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(With Plate 3)

A form of anthracnose, caused by *Gloeosporium musarum* Cke. & Massee, is responsible for appreciable wastage of Jamaican Lacatan bananas. Symptoms are often evident on immature fruit after 8–10 days storage in refrigerated ships' holds. On green fruit, lesions are characteristically lenticular, having a slightly sunken, dark centre and an orange-yellow border: they rapidly increase in size as the fruits approach maturity. The fungus readily infects small scratches on the skin and, in inoculation experiments, resulted in the development of lenticular lesions after 8–10 days storage at 55° F. Many observations suggest that naturally occurring infections often originate at small abrasions acquired during handling of fruit.

On the basis of histological findings, it is proposed to refer to lenticular lesions as 'non-latent anthracnose', thus distinguishing this form of infection from the 'latent' type described by earlier workers.

In several experiments the antifungal antibiotic nystatin, applied as a post-inoculation fruit-dip at concentrations of 200 and 400 p.p.m., effected good control of wound-infection by *G. musarum*. Percentage control was inversely related to the incubation period between inoculation and treatment, there being little or no control with periods exceeding about 30 hr.

Introduction

During recent years the quality of Jamaican bananas imported into the United Kingdom has declined markedly. A major factor contributing to this decline has been the development of fruit-rots during transport and storage. Preliminary surveys made in England revealed that *Gloeosporium musarum* Cke. & Massee was the organism most commonly associated with such rots. In some cases wastage caused by this fungus has affected the complete shipment, which may vary between 70,000 and 130,000 stems.*

The investigations described here were carried out in an attempt to formulate control measures for reducing the amount of *Gloeosporium*-rot, in particular anthracnose. Other forms of fruit-rot caused by *G. musarum* will be described elsewhere.

* *Note on terminology.* In this paper, the individual banana fruit is referred to as a 'finger' and its short stalk as the 'finger stalk'; several 'fingers' constitute a 'hand', which is attached to a 'cushion' projecting from the 'main stalk'. The whole bunch, or 'stem', normally has five or more 'hands'.

HISTORICAL

Banana anthracnose, also known as black-rot or ripe-fruit rot, has been recorded in most banana-growing regions. It is caused by *G. musarum* and was originally described by Cooke & Massee from Australia in 1873, and later by Halsted (1893) in the United States.

Although the disease has been observed in the Philippines on immature bananas (Agati, 1922) and in India (Dastur, 1916) and Ceylon (Park, 1930) on immature plantains, the symptoms usually become manifest on almost mature fruit. Agati (1922) demonstrated the ability of *G. musarum* to infect very immature fruit, but for several years investigators in the western tropics were unable to obtain similar results (Wardlaw, 1931). Dastur (1916) had suggested that infection is initiated in the field, remaining in a dormant condition until maturity ('latent infection' theory). Working in Trinidad, Baker & Wardlaw (1937) found that thoroughly surface-sterilized immature fruit, when allowed to mature in aseptic containers, developed typical anthracnose lesions. The work of Simmonds & Mitchell (1940) and Simmonds (1941) provided final confirmation of the latent-infection theory. Experimental inoculation of immature fruit showed that the infections remain superficial for a considerable period, resuming activity only at late stages of ripening. More recently, Chakravarty (1957) has shown that cuticular penetration is effected by mechanical means; a slender infection hypha, developed from an appressorium, lies in a subcuticular position until maturity is approached. Further penetration of tissues then occurs. It was also found that the expressed juice of green, but not of yellow, banana skin had an inhibitory effect on germination of conidia. This was considered to be the physiological basis of latency.

Delacroix (1902) considered *G. musarum* a wound parasite and unable to infect unwounded fruit. Although the researches of the above-named workers have disproved this contention, Wardlaw (1935) and Leach (1958) have emphasized the importance of mechanical injury as a factor favouring infection by *G. musarum*. However, these workers do not clearly differentiate the symptoms of latent infection from those of wound infection. This has been attempted in the following sections.

MATERIALS AND METHODS

Preliminary observations, made at ports of discharge and at banana-ripening stores situated in the United Kingdom, were designed to study the development and relative importance of various forms of fruit-rot caused by *G. musarum*. All observations and experiments were made on the Lacatan variety of banana, which constitutes more than 90% of the total banana export from Jamaica.

In Jamaica, two specially constructed storage chambers were used for experimentation. In the first, the refrigeration installation was adjusted to produce air temperatures ranging from 50 to 60° F. Both temperature and relative humidity were normally maintained at values corresponding to those inside the holds of banana ships, viz. for Lacatan fruit 55–57° F. and greater than 90% R.H. Experimental fruit was subjected to the above conditions for 10–12 days, this being the

average period in transit. Air temperatures in the second chamber were maintained between 64 and 70° F., according to the desired rate of ripening. At 68–70° F. the average time taken for fruit to attain full maturity was 4–5 days (fast ripening), while at 64–66° F., 6–7 days were required (medium ripening). At all temperatures a very high relative humidity (95–100%) was maintained until the fruit 'turned' from green to yellow: after this the value was allowed to remain at about 90%.

A difficulty commonly encountered with the Lacatan variety is uneven ripening of fruit. The introduction of ethylene gas at the rate of 1 cu.ft./1000 cu.ft. of ripening space was the best method of promoting even ripening.

Symptoms

Wardlaw & McGuire (1931) considered latent infection one of the most important sources of wastage of Gros Michel and Cavendish bananas. The present investigation has shown that it is of negligible significance on mature Jamaican fruit of the Lacatan and Gros Michel varieties. The few lesions observed became evident only at full maturity as small, more or less circular, fawn to light-brown diseased areas against the background of yellow skin: sectioning revealed the superficial nature of the lesions. At temperatures above 70° F. each lesion increased rapidly in diameter, becoming dark-brown or black. Where several lesions occurred on one finger, there was often extensive coalescence. At this stage the fungus had usually invaded the pulp to a depth of several millimetres, causing a watery rot. Large numbers of bright salmon-coloured acervuli developed on the surface of older lesions.

Of far greater importance as a source of wastage was another form of fruit-rot from which *G. musarum* was isolated consistently. Symptoms of disease were usually apparent on immature, green fruit at the time of arrival in the United Kingdom. The lesions were typically lenticular with the longest axis parallel to that of the finger (Pl. 3, fig. 1). Lesion dimensions, i.e. maximum length and breadth, on newly arrived fruit varied considerably, extreme examples being 6·0 × 1·2 cm. and 0·8 × 0·2 cm. Diseased areas were sunken and black in the centre, the lesion periphery being delimited from healthy green skin by an orange-yellow 'halo', as described earlier by Leach (1958). Acervuli were sometimes present on the necrosed tissue. In contrast to its behaviour in latent infections, the fungus had often penetrated the outer pulp tissues of green fruit. Rapid fungal spread occurred shortly after infected fruit was transferred to the relatively high temperature of the ripening room: in one instance, an increase in lesion length of 0·9 cm. per day was observed. On ripe fruit it was common to observe lesion dimensions up to 8·0 × 3·0 cm. The ratio of lesion length to maximum breadth, obtained by averaging measurements from twenty-five lesions, was 2·9, emphasizing the contrast between this form of infection and latent infection lesions which are characteristically more or less isodiametric. Diseased fingers mature faster than healthy ones, a condition known within the trade as 'patchy ripening' (Pl. 3, fig. 2): Wardlaw (1935) observed similar acceleration of maturation processes in fruit infected with other fungi. Final stages of rot are associated with extensive invasion of the pulp tissues causing a watery rot.

The observations described above contrast with that of Wardlaw (1931) who states that 'blemishes due to *Gloeosporium* do not become conspicuous until the fruit is approaching final maturity (i.e. yellowing)'. A possible explanation of this apparent discrepancy may derive from the fact that Wardlaw studied chiefly fruit of the Gros Michel variety: varietal susceptibility to infection by *G. musarum* will be discussed elsewhere.

WOUND INFECTION

Experimental and natural infection

An experiment was set up to provide information about the history of lenticular lesions. Apparently healthy, unwounded fingers, distributed among several newly harvested stems, were surface-sterilized by swabbing with 0·1% $HgCl_2$ in 70% alcohol and washing with sterile water. Four inoculation types were tested:

(1) The skin was bruised with a blunt wooden edge until the tissues assumed a water-soaked appearance: care was taken not to rupture the epidermal layer.

(2) The skin was pierced lightly with a sterile needle-point to produce a wound approximately 0·5 mm. in diameter and less than 0·5 mm. deep.

(3) As for (2) but larger (6 mm. diameter) and deeper (more than 0·5 mm.) wounds. Wounded fingers were inoculated by spraying with a suspension of conidia of *G. musarum* (obtained from infected bananas), adjusted to approximately 2×10^3 spores/ml.

(4) Unwounded fingers were inoculated by spraying with the same conidial suspension.

Controls for each series were not inoculated. Fruit was stored at 55–56° F. for 10 days and ripened at 68–70° F. for 5 days. The results are given in Table 1.

TABLE 1. *Experimental inoculation of immature bananas with* Gloeosporium musarum: *numbers of lesions developed* (*out of thirty*) *at maturity*

	Bruised	Scratched lightly	Scratched heavily	Unwounded
Inoculated	6	26	30	2
Un-inoculated	0	0	0	0

After 5 days ripening all lesions observed were of the lenticular type. From these results it is clear that either large or very slight scratches are readily invaded by the fungus and that scratches are more conducive to the development of disease than are bruises. The two infections on unwounded fruit possibly originated at small abrasions that were already present, but not evident at the time of inoculation. After 6 days storage the fruit was over-ripe and several lesions of the latent infection type had developed on inoculated but not uninoculated fingers. These lesions probably originated by infection of undamaged tissues, in the manner described by Simmonds (1941) and Chakravarty (1957).

Lesion development during cold storage was observed both on naturally infected fruit, which had been collected from banana-loading wharves, and on artificially

infected fruit. After 6–8 days at 57° F. a proportion of previously marked superficial abrasions or scratches became more evident due to the appearance of an orange-yellow border. The centre of each young lesion became sunken and black. Gradual enlargement took place during the remaining 2–4 days storage, resulting in typical lenticular lesions. Similar observations have been made on fruit in transit by Leach (1958).

From these observations, it seems likely that, under natural conditions, lenticular lesions often originate through infection of skin wounds, normally acquired during the various handling operations between harvesting and ripening. Circumstantial evidence supporting this conclusion has been obtained consistently during the course of investigation. Thus, fruit that had been transported carefully to the ripening room never developed lenticular lesions, while fruit transported in the normal manner, i.e. in trucks containing large quantities of rough leaf-trash, frequently developed lesions. Transport trials, using various types of padding materials, have provided further evidence (Leach, 1958). It has been found that the incidence of lenticular lesions is highest on the outer sides of the more exposed fingers of each stem (Meredith, 1959). This is probably due to these fingers suffering more mechanical injury than less exposed ones.

Pathological histology

Immature banana fruits were wound-inoculated with *G. musarum* and stored at 55 or 85° F. in closed 'Polythene' bags. Material for sectioning was fixed in formalin-acetic-alcohol and microtome sections 8–10 μ thick were stained with aniline blue in lactophenol. At the higher temperature, conidia germinate on injured tissues in 3–6 hr. Each conidium produces one or more germ tubes in the manner described by Chakravarty (1957). Germ tubes spread in all directions throughout the disorganized cellular tissue, and after 8 hr. may be up to 20 μ in length. It is unusual for appressoria to be produced, contrasting with the situation when conidia germinate on unwounded epidermis (Chakravarty, 1957). The approximate maximum extent of hyphal penetration, expressed in terms of numbers of healthy parenchyma cells traversed, was estimated in material that was fixed at various intervals after inoculation. After 8, 20, 30, 44 and 54 hr., observed values were 1, 2, 5, 8 and 12 cells, respectively. The fungus traverses the skin parenchyma by way of the middle lamellae and intercellular spaces: hyphae also pass from cell to cell, penetrating the cell wall, confirming earlier observations by Wardlaw (1931) and Chakravarty (1957). At later stages, the vascular tissues are invaded and hyphae were observed in the vessel lumina. In immature fruit, the fungus grows more rapidly along the lumina of xylem elements and latex tubes than in parenchymatous tissue. This may account for the lenticular form assumed by lesions originating from wound infections.

In one instance, a small acervulus was observed on material that was fixed after 44 hr. incubation at tropical temperatures. This would appear to be a relatively short generation period, for Chakravarty (1957) did not observe acervulus development until 116 hr. after inoculation. The morphology of acervulus development on

older lesions was similar to that described by Wardlaw (1931), except that conidiophores developed on the surface of injured tissues and not in a subcuticular region.

At the cold-storage temperature, the rate of fungal penetration is appreciably slower than at tropical temperatures. Thus, comparable extents of penetration were observed after 72 hr. at *c.* 85° F. and 120 hr. at 55° F. Further penetration is effected in the manner described above.

From these studies it is evident that *G. musarum* does not become dormant after infecting wounded tissues. Since the symptoms of this form of infection are readily distinguishable from those of the latent form, it is proposed here to refer to it as 'non-latent anthracnose'. Such a distinction may help to avoid confusion, for the term anthracnose has been used to describe any form of localized lesion infected with *G. musarum* (Castellani, Beccari & Matta, 1958). The evidence suggests that non-latent anthracnose is essentially a post-harvest wound infection, whereas latent infections are often initiated on unwounded fruit in the field (Simmonds, 1941; Chakravarty, 1957).

Chemical control of anthracnose

The possibility of controlling *Gloeosporium* infection by chemical means was investigated. For this purpose, a standard inoculation technique was employed. Apparently healthy and uninjured green fruit was inoculated by lightly scratching the skin to produce a circular wound approximately 2 mm. in diameter but less than 0·5 mm. deep. Wounds were sprayed immediately with a spore suspension of *G. musarum* (obtained from infected fruit) containing approximately 2×10^3 spores/ml. Selected chemicals were then applied in the appropriate manner. It is commercial practice to protect stems during shipment by enclosing them in perforated 'Polythene' tubing tied at each end of the main stalk. This form of wrapping was employed for experimental fruit that was stored in the cold chamber, the tubing being removed after transfer to the ripening room.

Fumigant fungicides

The discovery by Fawcett, Spencer & Wain (1958) of certain (aryloxythio)-trichloromethanes, with fumigant properties, suggested the possibility of fumigating bananas during storage in order to control fruit-rots.

Small-scale experiments on *Gloeosporium* fruit-rot were initiated using the 2:4:5-trichlorophenoxythio analogue. *In vitro* tests, carried out in the manner described by Fawcett *et al.* (1958), showed a marked fumigant action against *G. musarum*. The fumigant activity *in vivo* was tested by inoculating detached fingers and placing them in damp chambers. The compound was added as acetone solution to filter-papers at rates equivalent to 25, 50 and 100 mg./l., respectively, of air space in the containers: after evaporation of the solvent, filter-papers were fixed inside the containers. After 18 hr. storage at 57° F. it was evident that the compound had caused severe phytotoxicity, even at the lowest concentration used. In all treated fruit, the skin had darkened considerably, presenting a scorched

appearance: subsequently it was found that ripening was almost completely inhibited. Although there was some control of infection (45, 35 and 20% control at 100, 50 and 25 mg./l., respectively), it was obvious that the compound held little promise for commercial application to banana storage.

Cyclo*heximide* (*actidione*)

*Cyclo*heximide,* an antifungal antibiotic, has been used successfully for the control of cherry leaf spot (Cation, 1953; Hamilton & Szkolnik, 1953) and turf diseases (Howard & Davies, 1953).

In the present investigation the *in vitro* activity of *cyclo*heximide was tested on *G. musarum*. The minimum inhibitory concentrations, as determined by the spore germination and agar-streak dilution assays, were 2·5 and 5 p.p.m., respectively.

A preliminary experiment was designed to test the activity of *cyclo*heximide in controlling post-harvest infection by *G. musarum*. Aqueous solutions of the antibiotic, containing 2·5, 5, 10, 25, 50 or 100 p.p.m., respectively, were prepared and a wetter of the sodium secondary alkyl sulphate type added. Immediately after inoculation with *G. musarum*, fruit was dipped for 5–8 sec. in the antibiotic, wrapped in perforated 'Polythene' tubing and stored at 57° F. for 11 days. Controls were provided by fruit which was dipped in water plus wetter only. Observations were made after 7 days ripening at 68° F., when controls were fully mature. The recorded data showed that there was no control of infection at concentrations below 25 p.p.m., but at 50 and 100 p.p.m. there was approximately 15 and 40% control, respectively. However, above 25 p.p.m. there was severe blackening of the skin together with a marked inhibition of ripening: slight inhibition was apparent at 5 p.p.m. It was concluded that phytotoxic effects caused by *cyclo*heximide preclude its use for control of banana fruit-rots.

Mycostatin (*nystatin*)

'Mycostatin' is a preparation† of the antifungal antibiotic nystatin, present in the mycelium of *Streptomyces noursei* (Brown & Hazen, 1957). A high *in vitro* activity against *G. musarum* was observed by Rangaswami & Damodaran (1958). Castellani *et al.* (1958) have tested the compound as a post-harvest dip for fruit that was shipped unrefrigerated from Somaliland to Italy: preliminary trials suggested that there was some control of *G. musarum* infection, although the exact nature of infection was not specified.

In the present investigation, a more detailed study of the possibility of controlling *G. musarum* infections with nystatin was undertaken.

A preliminary experiment was designed to compare the effects of dipping fruit in nystatin at concentrations of 100, 200 and 400 p.p.m., respectively. Antibiotic suspensions were made in water, and a wetter of the secondary alcohol sulphate type was added. Although nystatin is insoluble in water, it is stated that alcoholic

* Samples were supplied by The Upjohn Company, Kalamazoo, Michigan.

† Prepared by E. R. Squibb and Sons, Division of Olin Mathieson Chemical Corporation, New Brunswick, New Jersey.

compounds generally have a reasonably good solubilizing effect (Mathieson Chemical Corporation—personal communication). One hour after inoculation, fruit was immersed in the dipping medium for 5–8 sec., enclosed in perforated 'Polythene' tubing and stored and ripened in the usual manner. Controls were dipped in water plus wetter only. The results obtained, based on the numbers of lesions which developed at inoculated points, showed that there was a significantly lower incidence of infection ($P < 0.05$) on nystatin-treated fruit (200 and 400 p.p.m.) than on water-dipped controls. There was no significant difference at the 100 p.p.m. level or between the two higher levels of treatment. At 400 and 200 p.p.m. there was approximately 33 and 25% control, respectively, relative to infection of fruit dipped in water plus wetter only.

In another experiment, two levels of antibiotic—200 and 400 p.p.m.—were compared with water as a post-inoculation treatment, carried out immediately or after 3 or 9 hr. incubation. A randomized lay-out with six replicate stems for each treatment was employed in order to reduce errors due to differing physiological conditions between stems and to environmental variation within the cold-storage and ripening chambers: on each stem, twenty fingers were inoculated. Experimental details were similar to those in the previous experiment except that after inoculation, some stems were incubated at 83–85° F. for 3 or 9 hr. in unperforated 'Polythene' bags prior to treating. The results of this experiment, and of two similar ones in which 400 p.p.m. of nystatin was tested, are summarized in Table 2.

TABLE 2. *Effect of nystatin on post-harvest infection by* Gloeosporium musarum*: percentage numbers of lesions (out of* 120*) developed following inoculation and treatment after various incubation periods*

Experiment	Treatment	Incubation period (hr.) 0	3	9	Mean
1	Water (controls)	87·5	83·8	52·9	74·8
	Nystatin (200 p.p.m.)	52·0	30·2	25·4	35·9
	Nystatin (400 p.p.m.)	38·5	21·8	21·8	27·4
	Mean	59·4	46·5	33·4	—
2	Water (controls)	64·6	63·9	49·3	59·3
	Nystatin (400 p.p.m.)	17·8	4·7	4·0	8·8
	Mean	41·2	34·3	26·6	—
3	Water (controls)	73·3	82·4	76·6	77·4
	Nystatin (400 p.p.m.)	52·4	35·8	32·5	40·2
	Mean	62·8	59·1	54·5	—

For each experiment, an analysis of variance was performed on the angular transformations of the recorded data. Over all incubation periods tested, the incidence of infection in nystatin-treated fruit was significantly lower ($P < 0.001$) than that in water-dipped controls: however, in Exp. 1, there was no significant difference between the two levels of antibiotic. From the above table it is seen that at 400 p.p.m. nystatin effected approximately 60, 45 and 80% control, respectively: at 200 p.p.m. there was approximately 50% control. The variance ratio for incubation period/error was significant in Exps. 1 and 2 ($P < 0.001$ and $P < 0.05$,

respectively) but not in 3. In the former cases the data for controls show a marked decrease in incidence of infection after 9 hr. incubation. This is probably accounted for by the fact that several inoculated wounds dried out appreciably owing to a low incubation humidity: measures were taken to rectify this in Exp. 3. The results from nystatin-treated fruit suggested that there was slightly better control when dipping was carried out after 3 or 9 hr. incubation. However, in Exps. 1 and 2, the low incubation humidity may have been partly responsible for this: even in Exp. 3, where the data for controls were reasonably uniform, the observed differences between variously incubated, nystatin-treated fruit are not significant.

The effect of incubation period on nystatin control was investigated further. Fruit was inoculated and then incubated at 85° F. for 0, 8, 16, 24, 32 or 40 hr. before being treated. For each type of treatment, i.e. dipping in water or nystatin, seven replicate stems were inoculated at staggered intervals in order to include all incubation periods on each stem. Data obtained after cold storage and ripening are summarized in Table 3.

TABLE 3. *Numbers of lesions developed (out of seventy) on fruit inoculated with* Gloeosporium musarum *and treated with nystatin or water after various incubation periods*

Treatment	Incubation period (hr.)						Mean
	0	8	16	24	32	40	
Nystatin (400 p.p.m.)	11	6	24	36	40	46	27·2
Water (controls)	62	44	56	59	50	54	54·2

There was good control of infection by nystatin when the incubation period did not exceed about 16 hr. Longer incubation resulted in a marked decrease in control. Analysis of the transformed data has shown that there is no significant difference between the data for treated and control fruit that was incubated for 32 or 40 hr. In these cases it must be concluded that there was no control of infection. Histological evidence suggests that, after about 30 hr. incubation, the fungus may have penetrated the tissues to such an extent that it escapes antibiotic action. Under commercial conditions, therefore, it would be desirable to minimize the interval between harvesting and treatment with nystatin.

It was shown earlier (p. 282) that typical lenticular lesions may develop from inoculated bruises or scratches, but that scratches are more favourable for infection than are bruises. An experiment was set up to compare the effect of nystatin on three inoculation types. Experimental details were similar to those already described, dipping in water, 200 or 400 p.p.m. nystatin being carried out after 3 hr. incubation. A randomized lay-out, with five replicate stems inoculated so as to provide a maximum possible number of seventy-five lesions for each treatment combination, was employed: all inoculation types were included on each stem. The results are shown in Table 4.

Statistical analysis of the transformed data once more showed a highly significant reduction ($P < 0{\cdot}001$) of infection due to nystatin: there was no significant

difference between the data obtained from the two concentrations of antibiotic. The variance ratio for inoculation type/error was also significant at the 0·1 % level and from Table 4 it is seen that large, deep scratches are more conducive to infection than superficial scratches or bruises. There was poor control of infection that originated in large scratches; it is possible that spores had lodged in a relatively deep-seated position in the mutilated tissues and so avoided antibiotic action. General experience has indicated that the majority of natural infections are more superficial than those induced experimentally, so that under commercial conditions reasonable control might be anticipated.

TABLE 4. *Numbers of lesions developed (out of seventy-five) on bananas inoculated in different ways with* Gloeosporium musarum *and treated with nystatin or water*

	Inoculation type			
Treatment	Bruise	Superficial scratch	Deep scratch	Mean
Nystatin (400 p.p.m.)	6	17	55	26·0
Nystatin (200 p.p.m.)	4	9	51	21·3
Water (controls)	10	55	70	45·0
Mean	6·6	27·0	58·7	—

TABLE 5. *Effect of various periods of exposure to tropical temperatures, prior to refrigeration, on control of* Gloeosporium musarum *by nystatin: numbers of anthracnose lesions developed (out of* 120) *on six replicate stems*

	Storage period at tropical temperature (hr.)			
Treatment	0	7	14	Mean
Nystatin (200 p.p.m.)	8	5	4	5·7
Nystatin (400 p.p.m.)	6	3	4	4·3
Water (controls)	55	43	55	51·0
Mean	23·0	17·0	21·0	—

Wardlaw & McGuire (1932) have shown that infections by fruit-rot fungi are established more rapidly at tropical temperatures than at hold temperatures (53–55° F.); they stressed the importance of minimizing the interval between reaping and loading fruit. On this basis it was considered desirable to investigate possible effects on nystatin control of different post-treatment exposures to tropical temperatures. Fruit was inoculated and dipped in water or nystatin (200 or 400 p.p.m.) after 3 hr. incubation. Each stem was then enclosed in a perforated 'Polythene' bag and stored at field temperatures (83–86° F.) for a chosen interval before transfer to the cold store (55° F.). The experiment was recorded when ripening was complete and the data obtained are given in Table 5. The results show that exposure of treated fruit to tropical temperatures for 7–14 hr. before cold storage does not markedly affect the efficiency of nystatin in controlling infection. This is of considerable practical importance since, under prevailing conditions in Jamaica, fruit may have to remain on the quay-side for several hours after nystatin treatment before being transferred to refrigerated ships' holds.

Further experiments were designed to test nystatin against naturally occurring *Gloeosporium* infections. Several stems were harvested from one field and transported over a short distance in trucks lined with banana leaf-trash. They were then segregated at random into two groups, one of which was dipped in nystatin (400 p.p.m.) and the other in water. After cold storage and ripening in the normal manner, the numbers of fingers with one or more lenticular anthracnose lesions present were recorded. Data obtained from two lots of fruit are shown in Table 6.

TABLE 6. *Effect of nystatin treatment on the incidence of natural infection of bananas by* Gloeosporium musarum

Experiment	Treatment	Total no. fingers examined	No. fingers with anthracnose	Percentage diseased fingers
1	Nystatin (400 p.p.m.)	1098	9	0·8
	Nystatin (200 p.p.m.)	1220	11	0·9
	Water (controls)	987	32	3·2
2	Nystatin (400 p.p.m.)	573	24	4·2
	Water (controls)	525	64	12·2

Although there was a relatively low incidence of infection on control fruit, the results from both experiments suggest that nystatin caused some reduction of disease.

In the experiments described above there was no evidence that nystatin was phytotoxic. However, at 400 p.p.m. there was a slightly slower rate of ripening compared with water-dipped controls. This sometimes resulted in fruit requiring an extra 24 hr. to attain full maturity. There was no apparent effect on ripening behaviour using 200 p.p.m. nystatin. Preparations have now been made for testing the compound on a commercial scale as a post-harvest dipping process carried out prior to stowage in refrigerated ships.

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EXPLANATION OF PLATE 3

(Photographs by Mr D. E. Aliband, Long Ashton Research Station.)

Fig. 1. Immature bananas with lenticular anthracnose lesions caused by *Gloeosporium musarum*.

Fig. 2. Fruit severely infected with *G. musarum* showing relatively advanced stage of maturity of diseased fingers (indicated by lighter shade).

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Fig. 1

Fig. 2

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(*Facing p.* 290)

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FIELD SPRAY TRIALS AGAINST WILDFIRE AND ANGULAR LEAF SPOT OF TOBACCO IN RHODESIA*

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Field tests in Southern Rhodesia compared several antibiotics with copper fungicides for the control of wildfire and angular leaf spot of tobacco.

When streptomycin-sensitive strains of the pathogen were used, streptomycin gave the best control. During one test, strains of *Pseudomonas tabaci* developed which were resistant to streptomycin: this was followed by a rapid breakdown of control. In another test, which compared streptomycin, oxytetracycline, neomycin, chlortetracycline and copper, streptomycin was the only effective spray. Cultures, isolated at the end of this test, showed resistance to all the antibiotics except chlortetracycline, but they lost their pathogenicity during laboratory tests. Oxytetracycline was generally no better than the lower rate of copper, and in one test it was ineffective. When variants resistant to streptomycin were used, a mixture containing oxytetracycline and streptomycin at quarter-strength and copper at half-strength was slightly better than full-strength copper or a mixture of half-strength copper and oxytetracycline.

Half-strength streptomycin and copper was slightly better than full-strength copper or half-strength streptomycin and oxytetracycline, against a mixture of streptomycin-resistant and sensitive variants of *P. tabaci*.

Introduction

Antibiotics have been tested in other countries for the control of wildfire of tobacco. Hitier & Izard (1955*a*, *b*), in France, found that streptomycin at 1000 μg./ml., when applied 12 hr. before inoculation, gave very good control but was phytotoxic, producing a chlorotic mottle; at 100 μg./ml. it was slightly better than 0·25% copper as oxide; aureomycin and terramycin were not very effective. Heggestad & Clayton (1954) controlled wildfire with streptomycin sprays and drenches at 100, 200 and 400 p.p.m.; these were considerably better than a Bordeaux mixture drench, or a 'fixed copper' drench or spray. Heggestad, Neas & Grosso (1956), Shaw & Thorne (1956) and Shaw, Lucas & Thorne (1957) reported that streptomycin sprays at concentrations of 200 p.p.m. gave better control than copper or terramycin. Sprays were generally better than dusts or drenches.

There have been many reports of the development in the laboratory of strains of phytopathogenic bacteria showing tolerance to antibiotics (English & Van Halsema, 1954; Carmona-Gomez, 1956). Dye (1958) has also isolated streptomycin-resistant variants of *Pseudomonas syringae* from peach seedlings sprayed with streptomycin. This paper describes some field experiments in which antibiotics and copper were

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used against *P. tabaci* and *P. angulata*, which cause wildfire and angular leaf spot of tobacco. In one experiment pathogenic cultures of *P. tabaci* were isolated, from sprayed seedlings, which were less sensitive to streptomycin than the culture used to infect them. In another, cultures, partially resistant to streptomycin, oxytetracycline and neomycin, were isolated, but these lost their pathogenicity during the sensitivity tests. Some of the resistant cultures were used in other field experiments to test mixtures of streptomycin, oxytetracycline and copper.

Materials and methods

Pure preparations of antibiotics of known potency were used for laboratory tests. Commercial preparations, when available, were used for field trials, otherwise pure antibiotics were dissolved in 0·1 % Lissapol.

Plants were inoculated, by spraying an aqueous suspension of bacteria forcibly on to the leaves, so that water-soaking occurred, 1 day after the first spray treatments were applied. Subsequent development of the disease was natural. Five trials were carried out with seedlings in seed-beds and two after they had been transplanted into the field. Unless otherwise stated the seed-beds were prefumigated with 1 lb. of methyl bromide/100 sq.ft. Sprays were normally applied at weekly intervals but occasionally this interval was reduced if rain fell soon after the sprays had been applied. Seedlings were assessed as having slight, medium or severe infection, or clean, and an infection index was calculated.

The paper disc method was used to assay the antibiotic content of juice. Leaves were washed for 1 min. in each of five changes of tap water, dried on blotting paper, wrapped in aluminium foil, frozen at $-5°$ C., thawed and the juice extracted through Polythene gauze and centrifuged. Paper discs, 13 mm. in diameter, were immersed in the juice for several seconds, dried at 37° C. for 2 hr. and placed on potato-dextrose agar previously seeded with *P. tabaci*. Inhibition zones were recorded as the mean distance from the edge of the disc to the region of growth, but only interpreted qualitatively. Sensitivity to the antibiotics was measured by the paper disc and serial dilution methods. The latter determined the range of concentrations, within which the minimum inhibiting concentration lay. All cultures were stored in sterile loam soil.

Results

In the first seed-bed experiment, a 13×4 Youden square was used with plots, 7×3 ft., containing a central sampling area $4{\cdot}5 \times 1{\cdot}5$ ft., from which all plants were pulled for assessment. The seedlings were inoculated with *P. angulata* and five treatment sprays were applied.

Streptomycin hydrochloride and the mixture of streptomycin sulphate and oxytetracycline gave good control of angular leaf spot and of a slight natural infection of wildfire; the copper preparations were not as effective (Table 1, first test). Zineb, maneb and thiram gave no control. Assays from samples taken from

the streptomycin treatments, 24 hr. after the second spray, showed that the antibiotic had been absorbed into the leaves (Table 2).

The same site was used for the second experiment, 2 months later; the soil was not resterilized. Procedure was similar except that sample areas were 3 × 1 ft., as in all subsequent seed-bed trials. Six sprays were applied. Seedlings were inoculated when 4 weeks old, with a recently isolated culture of *P. tabaci*. The initial

TABLE 1. *Control of wildfire and angular leaf spot of tobacco with sprays*

Material	% active ingredient	1st test, percentage of healthy seedlings (transformed to degrees)	2nd test, no. of seedlings per 3 sq.ft. transformed to log $(x+1)$ and adjusted for emerged seedlings	Infection index (transformed to degrees)
Control	—	46·0	0·40	—*
S.E.	—	(±1·44)	(±0·09)	—
Zineb	0·19	49·7	0·74	—*
Maneb	0·07	45·6	0·84	—*
Thiram	0·12	45·2	0·52	—*
Streptomycin sulphate+ oxytetracycline	0·02+0·002	77·0	1·86	50·3
Streptomycin sulphate+ oxytetracycline	0·04+0·004	76·4	2·06	48·6
Streptomycin hydrochloride	0·022	78·1	1·83	57·3
Streptomycin hydrochloride	0·044	81·6	2·05	61·7
Copper oxychloride	0·20	69·0	1·96	16·4
Copper oxysulphate	0·20	63·7	1·66	19·1
Bordeaux mixture	0·80	64·2	1·68	21·1
Cuprous oxide	0·15	68·3	—	—
Cuprous oxide	0·075	—	1·42	17·1
S.E.	—	(±2·04)	(±0·13)	(±0·38)
Least significant differences for comparisons between				
Control and treatments ($P = 0·001$)		9·4	0·62	—
Between treatments		10·8	0·72	2·1

* Omitted from the analysis because there were too few seedlings to give an accurate index.

TABLE 2. *Bioassay of antibiotics in tobacco leaves*

Material	Active ingredient (μg./ml.)	Inhibition zone (mm.) 1st test	Inhibition zone (mm.) 2nd test
Control	—	0*	—
Copper oxychloride	—	—	0*
Streptomycin sulphate+ oxytetracycline	0·02+0·002	0·1*	0·8
Streptomycin sulphate+ oxytetracycline	0·04+0·004	1·5	3·0
Streptomycin hydrochloride	0·022	0·8	0·8
Streptomycin hydrochloride	0·044	4·9	2·9
S.E.		(±0·48)	(±0·59)
Least significant differences ($P = 0·05$)		1·7	1·9

* Excluded from the analysis.

infection was very severe; during the subsequent week most of the seedlings died in all plots which had not received streptomycin or copper. Seedlings sprayed with streptomycin were more numerous and had less infection than those sprayed with copper. For a while this condition was maintained; later, although the copper plots improved and new growth was comparatively clean, seedlings sprayed with streptomycin became more heavily infected. The two phases of the experiment are shown in reference to Table 1, second test. The number of seedlings pulled, which included those recently dead, can be considered to represent the degree of protection initially given by the sprays; these data were transformed to log $(x+1)$ and adjusted for the number of seedlings originally present. The high rate of streptomycin gave significantly better protection than most of the copper sprays and was a little better than the low rate. Zineb, maneb and thiram were ineffectual. The infection indices show that the copper plots had much less disease, in the end, than the streptomycin plots and there was a slight reduction where oxytetracycline was present compared with streptomycin alone. It seemed either that streptomycin was not being absorbed, because of different growth or weather conditions, or that strains had developed which were less sensitive to the antibiotic. To test these hypotheses, samples were taken for antibiotic assay 24 hr. after the fourth spray and cultures were isolated for sensitivity tests. These tests showed that streptomycin was present in the juice (Table 2) and that the breakdown in control was caused by the development of pathogenic strains of *P. tabaci* having a low sensitivity to streptomycin, though still sensitive to oxytetracycline hydrochloride (Tables 3 and 4).

Table 3. *Sensitivity of strains of* Pseudomonas tabaci *by disc method*

	Inhibition zone (mm.)								
	Streptomycin hydrochloride			Streptomycin sulphate			Oxytetracycline hydrochloride		
μg./ml.	X3	A1	B2	X3	A1	B2	X3	A1	B2
256	5·9	0	0·5	13·3	0	0	14·3	15·2	12·7
128	5·1	0	0	11·5	0	0	13·5	14·5	11·0
64	4·6	0	0	11·0	0	0	10·4	11·9	9·1
32	3·8	0	0	9·2	0	0	8·0	9·9	6·9
16	3·2	0	0	7·5	0	0	6·4	7·9	6·1
8	2·6	0	0	6·0	0	0	4·9	6·4	4·2
4	1·3	0	0	3·9	0	0	2·0	4·0	3·3
2	0·4	0	0	2·0	0	0	0·6	1·0	0
1	0·1	0	0	3·3	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

X3, original inoculant.
A1 and B2, isolated from plants sprayed with streptomycin.

Another seed-bed experiment compared streptomycin sulphate with neomycin sulphate, oxytetracycline hydrochloride, chlortetracycline hydrochloride (aureomycin) and copper oxychloride, in an 11×5 Youden square. A freshly isolated culture of *P. tabaci* was used whose sensitivity to the antibiotics had been determined in liquid culture. Five sprays were applied.

Severe infection again followed the initial inoculation and a week later there were obvious differences between treatments. Full-strength streptomycin gave best control and full-strength copper and half-strength streptomycin were the only others that looked at all promising. The seedlings were assessed 3 weeks later; by

TABLE 4. *Sensitivity of strains of* Pseudomonas tabaci *by serial dilution method*

Culture	Inhibiting concentration (μg./ml.) Streptomycin sulphate	Oxytetracycline hydrochloride
P. tabaci X3	0·2–0·4	0·8–1·6
P. tabaci B2	32–64	0·4–0·8
P. tabaci A1	64–128	—

X3, original inoculant.
A1 and B2, re-isolated from seedlings sprayed with streptomycin.

TABLE 5. *Effect of several antibiotics and copper on wildfire in seed-bed*

Material	Active ingredient (μg./ml.)	No. of seedlings/sq.ft. Emerged	Surviving	Healthy
None	—	61·1	0·3	0
Copper oxychloride	1996	58·3	19·1	12·4
Copper oxychloride	998	49·4	5·1	1·0
Neomycin sulphate	440	55·8	0·7	0·1
Neomycin sulphate	220	63·1	0·8	0·2
Oxytetracycline hydrochloride	440	43·1	4·3	1·0
Oxytetracycline hydrochloride	220	55·8	1·4	0·3
Chlortetracycline hydrochloride	440	56·3	1·7	0·1
Chlortetracycline hydrochloride	220	61·7	1·4	0·1
Streptomycin sulphate	440	59·4	38·5	34·4
Streptomycin sulphate	220	54·3	14·5	12·1

TABLE 6. *Sensitivity of strains of* Pseudomonas tabaci *before and after spray treatments*

Culture	Inhibiting concentration (μg./ml.) Streptomycin sulphate	Oxytetracycline hydrochloride	Neomycin sulphate	Chlortetracycline hydrochloride
Original culture	0·2–0·4	0·1–0·2	0·2–0·4	0·8–1·6
Re-isolate 1	64	6·4	64	1·0–2·0
Re-isolate 2	64	6·4	64	1·0–2·0

then no deterioration had been observed in the streptomycin plots (Table 5), but cultures isolated at the end of the experiment were partially resistant to streptomycin, oxytetracycline and neomycin, though still sensitive to chlortetracycline (Table 6). Of two pathogenic cultures selected for sensitivity tests, one became weakly pathogenic and the other non-pathogenic. At the end of the sensitivity tests both were non-pathogenic.

Two other seed-bed experiments compared, in 13×4 Youden squares, streptomycin, oxytetracycline and copper, alone and in combination. In one experiment

four sprays of the materials under test were applied, and a new streptomycin-sensitive culture of *P. tabaci* was used. In the other, five sprays were applied and a streptomycin-resistant, but oxytetracycline-sensitive, strain was used (A 1 in Table 3).

Streptomycin was effective alone and with copper against the sensitive strain; no signs of resistance were observed. Oxytetracycline was completely ineffective alone but had a slight beneficial effect when combined with the other materials. Copper gave disappointing results, the lower rate being better than the higher. Unfortunately, no cultures were re-isolated during this experiment, but it seems possible either that resistance developed to oxytetracycline or that the original culture was not sensitive to this antibiotic.

TABLE 7. *Effect of sprays on two strains of* Pseudomonas tabaci

Effects of	Strepto-mycin (μg./ml.)	Oxytetra-cycline (μg./ml.)	Copper (μg./ml.)	Streptomycin-sensitive: % healthy seedlings transformed to degrees	Streptomycin-sensitive: Infection index	Strepto-mycin-resistant. Infection index
Streptomycin hydrochloride	—	—	—	38·84	23·8	61·1
	220	—	—	59·06	10·4	53·3
	440	—	—	73·64	3·0	66·8
	110	—	998	51·88	13·4	21·8
	220	—	998	67·94	6·1	24·9
Oxytetracycline hydrochloride	—	—	—	38·84	23·8	61·1
	—	408	—	35·24	28·3	27·7
	220	—	—	59·06	10·4	53·3
	220	204	—	53·84	13·9	37·7
	—	—	998	54·75	11·2	28·3
	—	204	998	51·15	13·7	17·8
Copper oxychloride	—	—	—	38·84	23·8	61·1
	—	—	998	54·75	11·2	28·3
	—	—	1996	48·48	15·4	16·5
	220	—	—	59·06	10·4	53·3
	220	—	998	67·94	6·1	24·9
	110	—	499	52·94	12·5	47·1
	110	—	998	51·88	13·4	21·8
All materials	110	102	998	60·80	9·1	11·4
Standard errors:						
Control	—	—	—	(±3·695)	(±2·61)	(±3·22)
Treatments	—	—	—	(±5·225)	(±3·68)	(±4·56)
Least significant differences (P = 0·001)						
Control and treatments		—	—	23·62	16·6	20·6
Treatments	—	—	—	27·27	19·1	23·8

Streptomycin alone was not effective against the resistant strain, but the lower rate was better than the higher, and mixtures of half- and quarter-strength with half-strength copper, were slightly more effective than copper alone. Cultures isolated at the end of the experiment showed no differences in sensitivity to streptomycin and had the same sensitivity as the original culture. Oxytetracycline in contrast to the results of the previous experiment reduced infection considerably.

At half-strength with half-strength copper, disease control was as good as with full-strength copper. Copper was effective at both rates. A mixture of all three constituents gave the lowest infection index.

The last two experiments were field tests; seven sprays were applied between transplanting and the start of reaping. A week after the last spray, all leaves were stripped and assessed. The weather was dry during both tests and infection was comparatively low. In the first (Table 8), two strains of *P. tabaci* were used, one sensitive and the other resistant to streptomycin and both sensitive to oxytetracycline. Equal concentrations of bacteria were used, determined by turbidity measurements. Results are expressed as numbers of infected leaves per plant because the leaves had very few spots. A half-strength mixture of streptomycin and copper was best, though full-strength copper was nearly as effective. Oxytetracycline, with streptomycin, was not as good as copper. Streptomycin alone significantly reduced infection.

The other field experiment tested copper and streptomycin at half- and full-strength and each was used at half-strength separately in the order, copper, streptomycin, copper, copper, streptomycin, streptomycin, copper, against a resistant

TABLE 8. *Effect of streptomycin, oxytetracycline and copper on a mixture of streptomycin-sensitive and streptomycin-resistant strains of* Pseudomonas tabaci

Material	Active ingredient (μg./ml.)	No. of infected leaves/ plant
None	—	4·8
S.E.	—	(±0·25)
Streptomycin hydrochloride	440	3·3
Streptomycin sulphate + oxytetracycline hydrochloride	220 + 202	1·8
Copper oxychloride	1996	1·1
Streptomycin hydrochloride + copper oxychloride	220 + 998	0·8
S.E.	—	(±0·35)
Least significant differences ($P = 0·001$)		
Control and treatments	—	1·0
Treatments	—	1·1

TABLE 9. *Effect of streptomycin and copper on a streptomycin-resistant strain of* Pseudomonas tabaci

Material	Active ingredient (μg./ml.)	Time of application (weeks after planting)	Infection index
None	—	—	25·3
Copper oxychloride	998	3, 4, 5, 6, 7, 8, 9	15·3
Copper oxychloride	1996		8·2
Streptomycin sulphate	220		27·5
Streptomycin sulphate	440		27·6
Copper oxychloride	998	3, 5, 6, 9	
or			17·0
Streptomycin sulphate	220	4, 7, 8	
S.E.			(±2·61)
Least significant difference ($P = 0·05$)			7·8

strain. Table 9 shows that full-strength copper gave the best control and four copper sprays at half-strength and three of streptomycin were as good as seven copper sprays.

Discussion

When streptomycin-sensitive strains of *P. tabaci* were present results agreed fairly closely with those of other workers; streptomycin at 440 μg./ml. gave better control than copper sprays, although at 220 μg./ml. it was generally no better. The effectiveness of copper varied in these tests and in those reported elsewhere, but it usually gave reasonable protection. Differences between streptomycin and copper might depend partly upon the frequency and degree of water-soaking, since this facilitates entry of the bacteria into the leaf (Clayton, 1936), and since streptomycin can be absorbed in greater amounts than copper. Oxytetracycline was never as good as copper and sometimes gave little or no control. Neither neomycin nor chlortetracycline gave encouraging results.

Although streptomycin was the only antibiotic which was better than copper, the disadvantage of resistance developing has to be considered. The effect of such an occurrence may be complete breakdown in control in a short time. Mixtures of antibiotics would not seem to be the answer because it appears that strains may develop which are resistant to all the individual spray components. Alternating sprays of copper and streptomycin might possibly be effective, but the high price of the antibiotic makes its use as a control measure for wildfire and angular leaf spot of tobacco unsatisfactory in Rhodesia at present.

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THE BIOLOGICAL CONTROL OF THE WATTLE BAGWORM, *KOTOCHALIA JUNODI* (HEYL.), BY A VIRUS DISEASE

II. LARGE-SCALE EXPERIMENTS

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To obtain further information about the effect of virus on wattle bagworm and on the course of an infestation cycle, suspensions of various concentrations and different origins were tested in 1957 against both young and old larvae. Virus of suspected low virulence and suspensions with and without adjuvants were also compared.

With infestations in the prodromal stage and the larvae in their first instar at the time of treatment, mortality was similar with suspensions containing 1000 or 500 polyhedra/mm.^{3}. Mortality was less with 250 polyhedra/mm.^{3}, but still high enough to keep the crowns of the infested trees green. With the higher concentrations, the course of the cycle was greatly accelerated and the eruptive stage and the crisis occurred later in the same season, whereas, with the lowest concentration, the infestation decreased gradually after spraying. Against older larvae, the initial mortality was too low to prevent defoliation, but 9 months after spraying the mortality had increased sufficiently to reduce the infestation to its latent stage.

Where virus was applied during the eruptive stage, there was a very high initial mortality, and defoliation of trees was light, whereas untreated trees were completely defoliated. Nine months after spraying, however, mortality was equally high in treated and untreated plantations.

Viruses from different sources differed considerably in the mortality they caused. Virus introduced from a distant source was more effective than local strains, and at the prodromal stage was more successful in preventing defoliation and loss of yield.

Methyl cellulose, used as a sticker, did not affect mortality, but skim-milk powder decreased it.

Artificially disseminated virus can not only cause epizootics in the treated plantations, but can also spread to neighbouring blocks in the years following treatment.

Introduction

The author (Ossowski, 1957) described pilot experiments made to see whether the wattle bagworm, *Kotochalia junodi* (Heyl.), could be controlled by artificially increasing the concentration of polyhedral virus in plantations, and whether epizootics could be initiated by introducing the virus. Aqueous suspensions of polyhedra sprayed on trees killed many worms, but the concentrations applied proved to be unnecessarily high, because the maximum mortality was reached at the lowest, viz. 10,000 polyhedra/mm.^{3}.

In 1956, further experiments (Ossowski, 1958*a*) with lower concentrations of virus from 1000 to 10,000 polyhedra/mm.^{3} showed no difference in the total kill at different concentrations. But the mortality was lower in plantations sprayed with

suspensions of local virus than in those sprayed with virus from a distant source, suggesting that the virus may occur in different strains (Ossowski, 1958*b*).

To obtain further information about the effect of virus on bagworms and on the course of an infestation cycle, plots covering over 500 acres in different parts of the wattle-growing area were sprayed from the air in 1957 (October–November). The experiments were designed to investigate:

(i) the influence of concentration and origin of the virus used against first-instar larvae (Exp. I(*a*));

(ii) the influence of origin against second/third-instar larvae (Exp. I (*b*));

(iii) the significance of virulence of the virus (Exp. II); and

(iv) the effect of adjuvants (Exp. III).

An unplanned deviation provided an opportunity to study the effect of virus on the course of an infestation during more advanced stages of the cycle (Exp. IV).

Material and methods

Aqueous suspensions evaporate quickly when sprayed over plantations from aircraft, and the dry deposit of polyhedra may then be carried far beyond its intended destination, especially when light cross-winds, favouring an even distribution of the spray, prevail during operations. Plots and buffers must, therefore, be much larger than when oil-borne insecticides are used. Further, it was essential that the plots should have approximately the same bagworm population density and stage of cycle and that the trees should be about the same age, stocking, and crown density. Because of these requirements and the nature of the plantations, it was not possible always to find plots for replicates, or even for the separate individual comparisons, in the same locality. But it was possible to duplicate all treatments except the one on Elim (Tables 3(*a*) and (*b*)), though some of the plots, notably Bloemendal and Welverdiend, and Tweedie and Bishopstowe, were as much as 15 miles apart.

All but one of the plantations were 3–5 years old and carried 550–600 trees per acre. The exception was Elim (Plot 2, Tables 3(*a*) and (*b*)), where the trees were 7–8 years old and the stocking approximately 450 trees per acre. The average size of the plots was 25 acres, though they varied somewhat due to the configuration of the ground and uneven population density. The canopy was dense and even throughout.

Before spraying started, the infestation had entered the first or second year of the prodromal stage, but at Albert Falls the infestation moved rapidly into the eruptive stage shortly after the commencement of hatching. In four experiments, bagworms were in their first instar at the time of spraying, and in one (Tables 2(*a*) and (*b*)), in the second or third instar.

Virus suspensions were prepared as already described (Ossowski, 1957). Some plots were sprayed with virus obtained from the same locality as the plots ('local') and others with virus obtained from remote plantations ('introduced'). In one experiment, a virus suspected of being of low virulence was used. Except in the

experiment to compare adjuvants, where suspensions containing 0·5% spray-dried skim-milk powder or no adjuvant at all were included, all suspensions contained 0·5% methyl cellulose (Celacol M. 1000). The concentrations of polyhedra used were 250, 500, 1000 and, in one experiment, 10,000/mm.^{3}, and the rate of application was 5 gal./acre where bagworms were in the first instar and 10 gal./acre where they were older. The aircraft was a 150 H.P. Piper Super Cub P.A. 18A equipped with forty-six spray nozzles with D8 orifices and with a tank holding 90 gal.

Rainfall records were kept from the first day of spraying to the end of April, when the larvae stopped feeding.

The criteria by which the results were assessed were

(i) the increase in mortality caused by spraying,

(ii) the consequent change in the degree of infestation in the same and in the following season, and

(iii) the effect on the infestation cycle.

This involved seven separate examinations of the plots, three to determine (i) and four to determine (ii) and (iii).

Mortality was first assessed 2 weeks after spraying, again after 6 months, when the larvae had stopped feeding and the males had pupated, and a third time 3 months later, when all the surviving bagworms had matured. Males emerge approximately 1 month before females become sexually mature, so they had already left the bags at the time of the third examination. Empty, fresh pupal cases protruding from the bags were, therefore, regarded as indicating that these had contained healthy males. In each assessment, from six to nine trees, depending on the size of the plots, were selected at random and all bags removed and examined, giving over 800 sample trees with a mean population of approximately 40 bags/tree and a total of approximately 35,000 bags. Virus was detected as already described (Ossowski, 1957).

The degree of infestation, using the scale previously described (Ossowski, 1956), was first assessed about the start of operations, secondly at the beginning of 1958, when defoliation could easily be detected, thirdly during the hatching period in the season following spraying, and fourthly at the beginning of 1959. These observations also provided the basis on which the stage of the cycle was assessed.

Spray applications and results

Spraying started on 14 October 1957, in plantations near Albert Falls, Natal. These plots were originally intended to form part of the experiment on concentration and origin, but the methyl cellulose adjuvant in this particular preparation was not properly dispersed and clogged the spraying nozzles, causing the plots to be unevenly covered. The clots of methyl cellulose blocking the nozzles and feed pipes contained up to ten times the concentration of polyhedral bodies in the original suspension. The plantations, therefore, were not only unevenly covered, but almost certainly received fewer polyhedra than was intended. For these reasons, the plots were replaced in this experiment by similar ones at Tweedie (Tweedie 2 and 3 in Table 1(*a*)). However, a routine check of the Albert Falls area 1 week later

revealed not only a high mortality in the sprayed plots, but a high natural mortality in the unsprayed control and the neighbouring plantations also. This indicated that the infestation in the whole district was moving rapidly from the prodromal into the eruptive stage, and the possibility of reaching the crisis during the same season could not be excluded. The opportunity was taken, therefore, to investigate the effect on mortality and on the course of an infestation of virus applied during the more advanced stages of a cycle.

Experiment I (Natal Midlands). Concentration and origin of virus

(*a*) *Application to first-instar larvae.* Suspensions containing 250, 500 and 1000 polyhedra/mm.^{3} of local and of introduced stock with methyl cellulose (0·5%) as adhesive, were applied on 15–18 October 1957, at the rate of 5 gal./acre.

At all stages, there were significant differences in the mean mortality due to virus according to whether 1000, 500, or 250 polyhedra/mm.^{3} had been applied (Table 1(*a*)). In each case, mortality increased with the passage of time, but the total mortality with the lower concentrations tended to catch up with that with the highest.

The mortality was consistently and significantly higher (by 4·0–18·6%) 2 weeks after spraying with introduced virus than with local virus. It remained higher (by 3·2–19·8%) some 6 months after treatment, but at 9 months the difference had almost disappeared (0·1–8·7%). The total mortality also was always higher up to 6 months after treatment, but not after 9 months (0·1–0·9%).

The consequent effect upon the degree of infestation, as measured by defoliation of trees, soon became apparent (Table 1(*b*)). In all plantations sprayed with suspensions containing 1000 or 500 polyhedra/mm.^{3}, the infestation 3 months after treatment was less severe in plots sprayed with introduced virus than in those sprayed with local virus. With 250 polyhedra/mm.^{3}, the difference was small, but still favoured introduced virus. However, few larvae hatched in September/October 1958, i.e. in the season following treatment, and the natural mortality was high in all plots, irrespective of whether they were sprayed with introduced or local material.

Virus suspensions appeared to hasten the development of the cycle. With 1000 or 500/mm.^{3}, it was evident 3–4 months after treatment that the eruptive stage and the crisis had been telescoped, and by January 1959 the infestation had returned to its latent* stage (Table 1(*b*)) except at Welverdiend, where it was on the downgrade. Few larvae hatched in this plantation in the season following spraying and it probably became reinfested from nearby heavily infested blocks. With 250/mm.^{3}, hatching in the following season was normal, but there was a high natural mortality and by January 1959 the infestation was on the downgrade.

In the unsprayed areas, the infestation became more severe, trees were defoliated, and in January 1959 the cycle had reached the first year of the eruptive stage.

(*b*) *Application to later-instar larvae.* Larvae in their second or third instar were treated on 27 and 28 November 1957, with 10 gal./acre of suspensions of local and introduced virus containing 10,000 polyhedra/mm.^{3} and 0·5% methyl cellulose.

* In Ossowski (1957) this stage was referred to as 'endemic or normal'.

TABLE 1 (*a*). *Effect of concentration and origin of virus on first-instar larvae of wattle bagworms.* (*Mortality*)

Plantation and plot (Natal Midlands)	Rainfall (mm.) In 7 days after spraying	Rainfall (mm.) To end of April 1958	Concentration/mm.[3] and origin of virus	Mortality (%) October/November 1957 (after 2 weeks) Virus	Other	Total	April 1958 (after 6 months) Virus	Other	Total	July 1958 (after 9 months) Virus	Other	Total
Tweedie 1	22·6	812·3	1000 local	40·0	31·7	71·7	54·1	26·0	80·1	70·0	29·1	99·1
Tweedie 1A	22·6	812·3	1000 introd.	53·1	29·2	82·3	60·9	29·2	90·1	75·1	24·6	99·7
Bloemendal 14B	23·7	827·0	1000 local	31·7	44·9	76·6	40·1	49·0	89·1	69·3	25·0	94·3
Bloemendal 14A	23·7	827·0	1000 introd.	50·3	40·6	90·9	59·9	32·2	92·1	70·1	24·3	94·4
Tweedie	22·6	812·3	Unsprayed	9·7	22·8	32·5	10·9	29·8	40·7	14·9	34·4	49·3
Bloemendal 26	23·7	827·0	500 local	27·4	32·8	60·2	33·9	44·6	78·5	41·0	49·0	90·0
Welverdiend	44·8	816·3	500 introd.	31·4	38·6	70·0	39·5	40·4	79·9	49·7	40·4	90·1
Ravensworth 76	82·3	1029·2	500 local	33·3	28·6	61·9	41·0	41·6	82·6	46·1	45·0	91·1
Ravensworth 76A	82·3	1029·2	500 introd.	42·3	31·0	73·3	46·3	43·3	89·6	49·9	42·1	92·0
Welverdiend	63·4	821·2	Unsprayed	6·5	29·8	36·3	12·1	31·0	43·1	20·8	31·1	51·9
Tweedie 2	27·6	812·3	250 local	18·2	31·9	50·1	25·1	43·2	68·3	32·6	56·3	88·9
Tweedie 3	27·6	812·2	250 introd.	24·1	56·4	80·5	29·5	56·4	85·9	36·9	52·5	89·4
Tweedie 4	27·6	812·3	250 local	16·1	27·6	43·7	21·0	33·8	54·8	39·9	46·1	86·0
Bishopstowe	23·7	827·0	250 introd.	20·1	27·9	58·0	24·2	37·6	61·8	40·0	46·9	86·9
Bishopstowe	23·7	827·0	Unsprayed	12·2	23·7	35·9	18·6	31·6	50·2	29·9	33·8	63·7

Mortality due to virus	Strains	Concentration	Time
Standard error of means	1·07	1·31	1·31
Significant difference of means ($P = 0{\cdot}05$)	3·20	3·92	3·92
Significant difference of means ($P = 0{\cdot}01$)	4·39	5·38	5·38

Two weeks after treatment, the kill was very small, in the plots sprayed with local virus little different from the unsprayed control (Table 2(*a*)). Total mortality was also low, only from 5·3 to 17·7% higher than in the control plots. But both total mortality and that caused by virus later rose rapidly, and 7 months after treatment the means were 94·4 and 69·9%, respectively. Local and introduced virus gave similar results.

There was no noticeable decrease in the degree of infestation 1 month after spraying and all plots were slightly defoliated (Table 2(*b*)).

Hatching during September/October 1958 appeared to be normal in the whole experimental area, but mortality in all plots treated in the previous season was high. Three months later, the population in the sprayed plantation had returned to its basic level and the trees remained green, whereas in control areas, where the infestation had entered the first year of the eruptive stage, the trees were severely defoliated.

Table 1 (*b*). *Degree and course of infestation of virus on wattle bagworms*

Plantation and plot (Natal Midlands)	Concentration/mm.^{3} and origin of virus	October 1957		January 1958	January 1959	
		Degree	Stage	Degree	Degree	Stage
Tweedie 1	1000 local	4–5	2nd prodromal year	3–4	0	Latent
Tweedie 1A	1000 introd.	4–5	2nd prodromal year	2	0	Latent
Bloemendal 14B	1000 local	3–4	2nd prodromal year	2–4	0	Latent
Bloemendal 14A	1000 introd.	3–4	2nd prodromal year	1–2	0	Latent
Tweedie	Unsprayed	4–5	2nd prodromal year	4–6	4–7	1st eruptive yr.
Bloemendal 26	500 local	3–4	1st prodromal year	1–3	0	Latent
Welverdiend	500 introd.	2–4	2nd prodromal year	1–2	0–2	Downgrade
Ravensworth 76	500 local	2–4	2nd prodromal year	1–3	0	Latent
Ravensworth 76A	500 introd.	2–4	2nd prodromal year	1–2	0	Latent
Welverdiend	Unsprayed	2–4	2nd prodromal year	3–5	4–6	1st eruptive yr.
Tweedie 2	250 local	4	2nd prodromal year	3	1–2	Downgrade
Tweedie 3	250 introd.	4	2nd prodromal year	2–3	1–2	Downgrade
Tweedie 4	250 local	4	2nd prodromal year	3	1–2	Downgrade
Bishopstowe	250 introd.	3–4	1st prodromal year	1–3	1–2	Downgrade
Bishopstowe	Unsprayed	3–4	1st prodromal year	3–5	3–6	1st eruptive yr.

Experiment II (Northern Natal). Virulence of virus

In some plantations, although virus has always been present during the past 6 years, mortality was small, which suggested the virus might be a strain of low virulence. To test this hypothesis, virus from these plantations was compared on plantations at Elim with another from a local source and with introduced virus (Table 3(*a*)). Concentrations of 250 polyhedra/mm.^{3} of all three, with an additional treatment of 500 polyhedra/mm.^{3} of introduced virus, were originally chosen, but on the day of application (21 October 1957), heavy rain was clearly imminent and these concentrations were increased to 500 and 1000 to compensate for the expected loss. Volume/acre and adjuvant were the same as in Exp. I(*a*). Over 28 mm. of rain fell within 12 hr. of the start of operations and the total rainfall 7 days after treatment was nearly 180 mm.

In the plot sprayed with local virus, the kill from virus 2 weeks after treatment was greatest with introduced virus and least with the virus suspected to be of low

TABLE 2. *Effect of origin of virus on second and third-instar larvae of wattle bagworms*

(*a*) Mortality

Plantation and plot (Natal Midlands)	Rainfall (mm.) In 7 days after spraying	Rainfall (mm.) To end of April 1958	Concentration/mm.^{3} and origin of virus	Mortality (%) Mid-December 1957 (after 2 weeks) Virus	Other	Total	April 1958 (after 4 months) Virus	Other	Total	July 1958 (after 7 months) Virus	Other	Total
Clan 1	12·1	972·8	10,000 local	2·3	28·9	31·2	49·0	40·1	89·1	69·1	24·0	93·1
Clan 1A	12·1	972·8	10,000 introd.	9·9	31·2	41·1	41·1	46·7	87·8	70·1	24·9	95·0
York	11·9	936·8	10,000 local	7·1	27·1	34·2	40·3	40·7	81·0	70·3	20·8	91·1
Bloemendal 11	21·3	645·6	10,000 introd.	10·1	33·5	43·6	39·8	47·9	87·7	70·2	28·1	98·3
York	12·4	931·4	Unsprayed	3·9	19·8	23·7	17·6	20·5	38·1	26·9	33·0	59·9

(*b*) Degree and course of infestation

Plantation and plot (Natal Midlands)	Concentration/mm.^{3} and origin of virus	Early December 1957 Degree	Stage	January 1958 Degree	January 1959 Degree	Stage
Clan 1	10,000 local	4	2nd prodromal year	4	0	Latent
Clan 1A	10,000 introd.	4	2nd prodromal year	4	0	Latent
York	10,000 local	3–5	2nd prodromal year	3–5	0	Latent
Bloemendal 11	10,000 introd.	4–5	1st prodromal year	4–5	0	Latent
York	Unsprayed	3–5	2nd prodromal year	3–6	4–6	1st eruptive year

TABLE 3. *Effect of origin and virulence of virus on first-instar larvae of wattle bagworms*

(*a*) Mortality

Plantation and plot (Northern Natal)	Rainfall (mm.) In 7 days after spraying	Rainfall (mm.) To end of April 1958	Concentration/mm.³ and origin of virus	Mortality (%) October 1957 (after 2 weeks) Virus	Other	Total	April 1958 (after 6 months) Virus	Other	Total	July 1958 (after 9 months) Virus	Other	Total
Elim 1A	179·8	881·9	500 introd..	21·1	28·8	49·9	39·9	35·9	75·8	71·3	18·8	90·1
Elim 1B			500 introd. (low virulence)	10·0	6·9	16·9	21·2	38·9	60·1	36·1	34·8	70·9
Elim 2			500 local	14·9	7·0	21·9	30·1	38·8	68·9	69·0	20·1	89·1
Elim 3			1000 introd.	26·9	30·4	57·3	61·1	32·9	94·0	75·9	23·8	99·7
Elim			Unsprayed	6·0	5·4	11·4	8·6	11·0	19·6	10·6	17·0	27·6

(*b*) Degree and course of infestation

Plantation and plot (Northern Natal)	Concentration/mm.³ and origin of virus	October 1957 Degree	October 1957 Stage	January 1958 Degree	January 1959 Degree	January 1959 Stage
Elim 1A	500 introd.	3–5	1st prodromal year	2	0–2	Downgrade
Elim 1B	500 introd. (low virulence)	3–5		2–4	2–4	Probably on downgrade
Elim 2	500 local	3–5		2+	*	*
Elim 3	1000 introd.	3–5		1	0(–1?)	Latent
Elim	Unsprayed	3–5		4–5	4–6	2nd prodromal year

* Plantation felled in December 1958.

virulence. The total mortality was also less with local virus than with introduced, and less with the suspected low virulent than with the local virus (Table 3(*a*)). Nine months after treatment, there was no difference between local or introduced stock in either total mortality or that caused by virus infection, but in the plot sprayed with virus of a suspected low virulence, mortality from virus infection was 32·9 and 35·2% lower and total mortality 18·2 and 19·2% lower than with local and introduced virus, respectively.

With the suspensions containing 1000 polyhedra/mm.^{3}, the mortality from virus infection 2 weeks after spraying was 5·8% higher than where the same virus strain was used at 500/mm.^{3}, and total mortality was 7·4% higher. Nine months later, the differences were 4·6 and 9·6%, respectively.

A notable feature of this experiment was the greatly increased mortality after 2 weeks from causes other than virus in the plots where introduced virus was used. This was apparent, though less so, in some other experiments and the difficulty in identifying light virus infection with certainty after only 2 weeks may explain it. Undetected infection may have caused or contributed to some of the deaths recorded as 'other causes'. Because the virus is much more readily detected at later stages, this could also explain the fact that the kill attributed to other causes was often lower after 9 months than after 2 weeks.

Three months after spraying, the degree of infestation had fallen sharply (from 3–5 to 2+ and 2) where local virus or the lower concentration of introduced virus was used, and still further (3–5 to 1) with the higher concentration of introduced virus (Table 3(*b*)). It decreased also, though less (from 3–5 to 2–4), in the plantation sprayed with virus of suspected low virulence.

Because of drought, hatching during September/October 1958, was somewhat late; it was light in all three plots sprayed with 500/mm.^{3} and negligible in that sprayed with 1000/mm.^{3}. A survey in January 1959 showed less infestation (0–2) in the plot sprayed with introduced virus. In the plot treated with virus of suspected low virulence, the degree of infestation remained at 2–4, but the course of the cycle was probably on the downgrade; with the introduced virus it certainly was. The plantation treated with virus of local stock was felled in December 1958, after which infestation could not be assessed, but as the bark is not readily removed from defoliated trees, and these trees could easily be stripped, the infestation was probably light. With 1000 polyhedra/mm.^{3}, the population returned to its basic level during the 1958–9 season.

In the unsprayed plot, the infestation increased considerably during the season of treatment and damage to trees became evident. The following season, many larvae hatched and the infestation entered the second year of the prodromal stage.

Experiment III (*Pondoland*). *Effect of adjuvants*

Methyl cellulose or spray-dried skim-milk powder (0·5%) was added to suspensions containing 250 introduced polyhedra/mm.^{3} and compared with a similar suspension without addition. Five gal./acre was applied on 27 October 1957.

TABLE 4. *Effect of introduced virus at 250/mm.³ with different adjuvants on first-instar larvae of wattle bagworms*

(*a*) Mortality

Plantation and plot (Pondoland)	Rainfall (mm.) In 7 days after spraying	Rainfall (mm.) To end of April 1958	Adjuvant	Mortality (%) October 1957 (after 2 weeks) Virus	Other	Total	April 1958 (after 6 months) Virus	Other	Total	July 1958 (after 9 months) Virus	Other	Total
Brownhill 1	30·5	716·3	Milk	10·8	21·5	32·3	19·3	42·7	62·0	29·1	42·0	71·1
Brownhill 2	30·5	716·3	Methyl cellulose	24·3	27·4	51·7	39·4	35·0	74·4	49·4	40·0	89·4
Cathill	30·5	716·3	Milk	8·9	21·0	29·9	16·1	41·8	57·9	24·3	39·5	63·8
Dapper L.	30·5	716·3	Nil	26·6	29·7	56·3	39·0	40·0	79·0	56·9	33·4	90·3
Balls-up 1	35·0	796·5	Nil	26·3	26·3	52·6	31·5	45·0	76·5	52·1	37·8	89·9
Balls-up 2	35·0	796·5	Methyl cellulose	27·1	23·8	50·9	29·9	44·7	74·6	50·0	40·0	90·0
Balls-up	30·5	716·3	Unsprayed	6·7	13·2	19·9	8·7	27·2	35·9	10·9	34·7	45·6

(*b*) Degree and course of infestation

Plantation and plot (Pondoland)	Adjuvant	October 1957 Degree	October 1957 Stage	January 1958 Degree	January 1959 Degree	January 1959 Stage
Brownhill 1	Milk	2–3	2nd prodromal year	2–3	2	Difficult to assess
Brownhill 2	Methyl cellulose	2–3	2nd prodromal year	0–1	1	Downgrade
Cathill	Milk	2–3	2nd prodromal year	2	2	Difficult to assess
Dapper L.	Nil	3–4	1st prodromal year	2	0–1	Downgrade
Balls-up 1	Nil	2–4	1st prodromal year	1–2	1 or 1–2	Downgrade
Balls-up 2	Methyl cellulose	2–4	1st prodromal year	1–2	1 or 1–2	Downgrade
Balls-up	Unsprayed	2–3	2nd prodromal year	4	4–5	1st eruptive year

TABLE 5. *Effect of origin of virus on first-instar larvae of wattle bagworms in the more advanced stages of an infestation cycle*

(*a*) Mortality

Plantation and plot (Natal Midlands)	Rainfall (mm.) In 7 days after spraying	Rainfall (mm.) To end of April 1958	Concentration/mm.^{3} and origin of virus	Mortality (%) October 1957 (after 2 weeks) Virus	Other	Total	April 1958 (after 2 weeks) Virus	Other	Total	July 1958 (after 9 months) Virus	Other	Total
Albert Falls 1	22·1	970·5	250 introd.	45·1	36·0	81·1	69·8	28·0	97·8	88·9	10·8	99·7
Albert Falls 1A			250 local	39·2	38·3	77·5	58·9	37·0	95·9	80·1	17·9	98·0
Albert Falls			Unsprayed	12·9	38·2	51·1	20·1	66·9	87·0	29·9	68·2	98·1

(*b*) Degree and course of infestation

Plantation and plot (Natal Midlands)	Concentration/mm.^{3} and origin of virus	October 1957 Degree	October 1957 Stage	January 1958 Degree	January 1959 Degree	January 1959 Stage
Albert Falls 1	250 introd.	3–5	2nd prodromal year	1–3	0	Latent
Albert Falls 1A	250 local	3–5		1–3	0	
Albert Falls	Unsprayed	3–5		4–6	0	

The outcome of these trials confirmed earlier results (Ossowski, 1959). Milk powder interfered with the even distribution of the spray by intermittent blocking of the nozzles and the mortality (Table 4(*a*)) from virus infection in plots sprayed with suspensions containing milk was lower throughout (by 14·9–22·6%) than in the other treatments. The kill in plots where methyl cellulose was added was very slightly, and probably not significantly, lower than with no addition.

In plots sprayed with suspensions without adjuvant or with methyl cellulose, the infestation decreased 3 months after treatment (Table 4(*b*)), whereas in those treated with milk, it decreased slightly in one and remained at the same level in the other. In the unsprayed plantation, the infestation increased from light (1–3) to moderate (4–5).

Hatching in September/October 1958 appeared to be normal in all plantations, though in January 1959 the degree of infestation (Table 4(*b*)) in those sprayed with an addition of milk was higher than in the remainder and the course of the cycle at that stage was somewhat difficult to assess. The course of the cycle in plantations sprayed without an adjuvant or with methyl cellulose was on the downgrade. In the control plantations, the infestation had entered the eruptive stage.

Experiment IV (Natal Midlands). Stage of cycle

The experiment at Albert Falls, which was designed to test the effect of concentrations and origin of virus but abandoned because the spraying proved unsatisfactory, was recorded after the infestation in this area was seen to be moving rapidly from the prodromal towards the eruptive stage and there was a possibility that the crisis might be reached during the season.

Local and introduced viruses at 250 polyhedra/mm.^{3} had been applied with methyl cellulose on 14 October 1957, but the nozzles clogged and the concentration actually reaching the trees was uncertain. Nevertheless, mortality from virus, as well as total mortality, were high 2 weeks after spraying (Table 5(*a*)), and total mortality reached a mean of 98·8% after 9 months. Mortality in the unsprayed plot was also high, 51·1% after 2 weeks and 98·1% after 9 months, but death from infection was only 12·9 and 29·9%, against means of 42·1 and 84·5% in the sprayed plots.

In January 1958 the infestation reached the eruptive stage (Table 5(*b*)), which was followed immediately by the crisis, but the higher initial mortality in the sprayed plots meant that defoliation was light with the degree of infestation 1–3 compared with 4–6 in the control plot, which was partially defoliated.

Very few bagworms hatched during September/October 1958, and a survey in January 1959 showed that the infestation in sprayed and unsprayed areas had returned to its latent stage.

Discussion

When larvae were in their first instar and the infestation in the prodromal stage, differences in the mortality with virus suspensions containing 1000 or 500 polyhedra/mm.^{3} were slight. Concentrations of 250 polyhedra/mm.^{3}, however, gave

a lower kill, but mortality was still enough to keep the crowns of the trees green. With the higher concentrations, the infestation passed directly into the crisis in the year of operations and the bagworm population returned to its basic level the following season. With the lowest concentration, the infestation decreased after operations, whereas in control plantations it entered the first year of the eruptive stage and in the following season was still on the upgrade. Where, however, suspensions containing nominally 250 polyhedra/mm.^{3} were applied when the cycle was about to enter the eruptive stage and population density was very high, mortality was rapid and there was no defoliation. The concentration actually applied at this stage was probably lower than 250 polyhedra/mm.^{3}, and the satisfactory kill suggests that the concentrations of virus needed to control the pest decrease as the cycle progresses.

The difference between the effect of introduced, local, and possibly low virulent viruses confirms earlier ideas that there may be different strains of the nuclear polyhedral virus of *K. junodi*. The effect of introduced virus in decreasing defoliation suggests that such suspensions could prevent loss of increment of bark and timber, and perhaps that heavily infested plantations due for felling in the year of spraying could be stripped of bark during that season. Though applications of virus at a later stage of development of larvae seem to kill too few larvae to save increment, they may hasten the development of the course of an infestation cycle and end it prematurely.

It is doubtful whether the very high mortality attained by some of the treatments is necessary or desirable. Bird (1955) suggests that it is not advisable to destroy all insects, because infestations are controlled eventually by epizootics resulting from small introductions of the virus. Thus, live infection centres should be maintained to carry the infective agent from season to season. This may have occurred with the low concentration of 250 polyhedra/mm.^{3}. Nine months after operations, the total mortality was some 88% and the infestation on the downgrade. It did not, however, reach the basic level, and some 12% of insects were left alive to carry over the infective agent.

Bird's suggestion appears to be based on the bionomy of sawflies and possibly of some lepidoptera that are basically different from the psychids in their habits. With these insects, pupation takes place in the soil, and the majority of moribund or dead larvae drop to the forest floor, thus making it more difficult for the infective agent to be spread through a plantation. The bionomy of the wattle bagworm is different. Bags remain on the trees for several years, disintegrating slowly, and when they contain dead larvae, pupae, or adult females, these too break up slowly and fall from the disintegrating bag. If they are virus-infected, virus particles are thus dispersed by wind through the plantation. For this reason, it may not be necessary to keep live infection centres, though perhaps a combination of such centres and those provided naturally in wattle plantations may prove advantageous. Fully grown, virus-infected larvae produce more polyhedral bodies than first-instar larvae and where spreading the infection is the objective, it may be desirable to use low concentrations and to apply them when larvae are in their

later instars, thus allowing the larvae to reach the fourth or fifth instar before succumbing.

A gradual spread of the effect of virus was evident in plantations sprayed in 1955 and checked in 1956 (Ossowski, 1959). These plantations, as well as others treated in 1956, when examined in 1957 and 1958 were free from bagworms. Infestation was negligible in plantations bordering directly on those sprayed, but its severity increased in blocks further away. It seems likely that virus is spread naturally in wattle plantations by ichneumon parasites and predacious shield bugs. Birds, and possibly rodents, feeding on infected bagworms may also transmit virus particles through their droppings and so distribute them over long distances. Thompson & Steinhaus (1950) found that braconids are probably important in establishing foci of infection by mechanical transmission of the virus on the ovipositor, and Bird (1955) showed that scavenging insects transmit virus. Virus of the European sawfly, *Neodiprion sertifer* (Geoffr.), remained infective after passing through the alimentary system of a predacious shield bug, *Rhinocoris annulatus* L., and of the Continental redbreast, *Erithacus rubecula* L. (Franz, Krieg & Langenbuch, 1955). Bird also found that virus particles ingested by the cat-bird, *Dumetella*, and the cedar waxwing, *Bombycilla*, together with virus-infected larvae of *Neodiprion sertifer*, remained highly infective after being stored for 8 months at 40° F.

The efficacy of the virus seems to be little affected by rain during or after spraying. Over 28 mm. of rain fell during spraying and within 12 hr. after completion of operations at Elim (Table 3(*a*)), and the rainfall there 7 days after operations was nearly twice as high as at Ravensworth and eight times as high as at Bloemendal (Table 1(*a*)), where the same rate of application, concentration, and adjuvant were used. Nevertheless, the total mortality at Elim, though somewhat lower 2 weeks after spraying, was approximately the same as at Ravensworth and Bloemendal 9 months after operations. There seems little danger of the polyhedra on the leaves being washed off by rain, possibly because the leaves are rough and hairy.

Methyl cellulose used as an adhesive did not increase the mortality, and skim-milk powder decreased it, partly because of the nozzles clogging. Bagworm larvae are almost static when not overcrowded (Ossowski, 1957), and with unevenly distributed spray may not have eaten leaves sufficiently contaminated by virus to cause death. Clogging of nozzles by undispersed methyl cellulose in the Albert Falls plantations (Tables 5(*a*) and (*b*)) did not impair the efficacy of the suspensions, but the infestation cycle there had reached the eruptive stage, and the bagworms were overcrowded. Starving bagworms in these overpopulated plantations move from branch to branch in search of food (Ossowski, 1958*a*) and would be more likely to eat contaminated leaves. There may also be other reasons for the partial failure of suspensions with skim-milk powder. Suspensions sometimes had to be left standing overnight and may have fermented, when the virus particles may have been occluded or adsorbed, and thus inactivated by the acid-coagulated protein.

The experiments gave promising results, but they have produced more problems than they have solved. There is an evident need for further work on strains, and the significance of latent infection and of resistance of bagworm to virus call for

investigation. The optimum dose at various stages of the infestation cycle, the seasonal time of treatment, and the duration of the effectiveness of a single application also remain to be determined.

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THE USE OF ENDRIN AND OTHER INSECTICIDES AGAINST LETTUCE ROOT APHID, *PEMPHIGUS BURSARIUS* L.

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Several insecticides have been used as protectants against lettuce root aphid, including, in 1955, lindane, schradan, aldrin and endrin; the last-named giving the most promising results. With a dressing of an equal weight of 50% endrin dust added to lettuce seed, 86% reduction in aphid numbers was obtained. In 1957, a seed dressing with endrin also gave a good control, which was superior to that obtained with thimet 44D. However, in 1956 and 1958 endrin seed dressing failed to control the aphid. When a seed dressing with endrin was supplemented by an endrin spray applied to the seedlings a consistently good control resulted.

A high level of aphid control was obtained in 1958 with two sprays of diazinon applied along the lettuce rows, the first applied after singling and the other at the early rosette stage. Diazinon or endrin, applied to the soil at 4 lb. actual insecticide/acre and rotavated into the top 3 in. before sowing, also gave high levels of control.

No recommendations for the use of endrin on lettuce can be made until levels and persistence of residues are known.

The lettuce root aphid, *Pemphigus bursarius* L., feeds on the roots of lettuce, producing stunted plants with yellowing leaves or causing the plants to wilt and, in severe cases, to die. When indications of attack first show in the foliage, damage has already taken place and the aphids are so numerous on the roots, and so widely distributed throughout the soil, that effective control measures at this stage are no longer practicable. Therefore, in the present investigations, all insecticides were applied in anticipation of an attack by the aphid.

Annually from 1955 to 1958, a field experiment has been carried out at Wellesbourne against *P. bursarius*, using seed treatments and making insecticide applications to the lettuce plants before the main migration of the aphid from poplar.

1955 Experiment

Before seed dressings were used on lettuce in the 1955 field experiment, germination tests showed that the application to seed of more than 10% of its weight of a 75% lindane dressing was phytotoxic. However, with dressings containing 40% aldrin or 50% endrin, no phytotoxicity resulted when equal weights of dressing and seed were used. The germination of seed soaked in 0·8% schradan for 1 hr. was found to be unaffected.

The treatments used in each of the three randomized blocks of the 1955 experiment are shown in Table 1. The plots (10 × 5 ft.) were 3 ft. apart and the lettuce was drilled and singled to 1 ft. spacing between and within the rows. Resin-alcohol

TABLE 1. *Treatments, aphid populations and plant damage assessments in experiment for the control of* Pemphigus bursarius *on lettuce, Bungalow Field, Wellesbourne, 1955*

Treatment no.	Insecticide and formulation	Concentration (%)	Application rate	Aphids on twenty-four plants: On foliage, 1 July: *P. bursarius alatae* Total	*P. bursarius alatae* No. dead	Other lettuce aphids	On roots 26 July *P. bursarius*	Plants showing symptoms of damage (% of total): 6 Aug. Wilting	10 Aug. Wilting	10 Aug. Dead or dying
1	Lindane seed dressing	75·0	10 % of wt. of seed	13	2	71	12,810	42·8	35·4	10·4
2	Aldrin seed dressing	40·0	100 % of wt. of seed	23	9	105	6,480	25·2	32·3	4·0
3	Endrin seed dressing	50·0	100 % of wt. of seed	16	8	93	1,600	6·4	8·3	0·0
4	Schradan steep	0·8	Steeping for 1 hr.	12	1	109	14,820	29·7	30·0	12·8
5	Lindane dust	2·0	¾ oz./40 row ft. B.S.	7	0	62	9,240	38·8	37·4	6·3
6	Lindane spray	0·02	8 fl. oz./40 row ft. B.S.	16	6	72	11,160	52·3	50·9	8·8
7	Endrin spray	0·1	8 fl. oz./40 row ft. B.S.	4	1	112	8,400	32·2	37·1	5·1
8	Lindane spray	0·02	8 fl. oz./40 row ft. A.S.	11	1	67	16,240	56·6	43·9	24·4
9	Endrin spray	0·1	8 fl. oz./40 row ft. A.S.	9	3	96	3,730	21·1	29·8	1·0
10	Schradan spray	0·1	1 pt./40 row ft. E.R.	14	13	29	4,600	21·3	44·0	0·0
11	Nil (control)	—	—	10	3	130	11,780	39·0	49·5	13·3

B.S.—before singling (2–3 true leaves); A.S.—after singling (3–4 true leaves); E.R.—early rosette (11–12 true leaves).
For numbers of *P. bursarius* on the roots on 26 July L.S.D. between treatments– ($P = 0{\cdot}01$) = ±8,420; ($P = 0{\cdot}05$) = ±5,790.

sticker was used with all seed dressings and all seed treatments were done immediately before the seed was sown. The lindane dust was applied through a muslin bag shaken along the rows, precautions being taken to prevent the dust drifting on to adjoining plots. The sprays, proprietary emulsifiable solutions diluted in water, were applied with a small hand-pressurized sprayer held so that a 4 in. band of spray was delivered along the row. The lettuce was singled at the three to four leaf stage and the schradan spray treatment was applied on 30 June, the day that spring migrants of *P. bursarius* began to arrive in the crop.

On 1 July, the foliage of eight plants in each plot was examined for aphids (*P. bursarius* and others, chiefly *Nasonovia ribis-nigri* Mosley) and 26 days later the root systems of the same plants were sampled for *P. bursarius*. In this sampling a 6 in. core was obtained centrally over each root system, the eight cores from each plot being bulked and the aphids extracted by flotation in water. The aphid numbers found in both the foliage and the root examinations are given in Table 1, together with the percentages of plants showing foliar symptoms of attack. These percentages were derived from examination of every plant in each plot. On 10 August, when plants were recorded as dead or dying, those which showed lack of 'resilience' to the touch but no marked sign of collapse were graded as wilting.

The table shows that endrin seed dressing was the most effective treatment and reduced the aphid numbers on the roots by 86% as compared with the untreated plots. Sprays of both endrin and schradan (treatments 9 and 10) also caused significant reductions of aphid populations on the roots.

It will be noticed that the percentage of plants that showed foliar symptoms following root aphid attack is closely associated with the number of aphids recorded. Thus a fairly good estimate of the relative efficiencies of the treatments could have been obtained solely from an examination of the aerial parts of the plants.

The numbers of *P. bursarius* alatae found dead on the foliage on 1 July are not necessarily correlated with treatment, for when the count was made some of the alatae may not have been on the foliage sufficiently long to have been affected by insecticide, whilst some of those found dead had almost certainly died naturally. However, the high mortality of alatae of *P. bursarius*, together with the low incidence of other aphids in the case of treatment 10, shows that schradan markedly reduced the numbers of aphids on the foliage. The significant reduction in aphid numbers on the roots given by this treatment probably resulted from the killing of *P. bursarius* on the foliage, because schradan, applied as a top spray, would not have been translocated into the roots. Young aphids produced on the foliage by spring migrants of *P. bursarius* insert their stylets into the leaves before moving down on to the roots (Dunn, 1959) and should they feed when so doing, their numbers also would have been reduced by the schradan treatment.

A second series of 6 in. soil cores was taken on 11 August from the plots of treatments 3, 9, 10 and 11. The aphids were extracted by flotation and because of the large numbers involved they were counted, as in the first series, by using the grid described by Strickland (1954). The accuracy of this method with *P. bursarius* was found to be very high.

To simplify counting further, the aphids in the second series were washed over a sieve of 30 meshes/in., and only individuals which were retained by the mesh were counted. The numbers passing through this mesh were between 30 and 40% of the total and, whilst they were too small to have done much damage to the host plants, their presence in a sample added greatly to the task of counting.

The aphid numbers for each treatment in the second series of root samples are given in Table 2.

TABLE 2. *Populations of* Pemphigus bursarius *on the roots of lettuce* 11 *August* 1955

Treatment	No. of aphids on 24 plants (to nearest 100)	
3. Endrin seed dressing	31,500	
9. Endrin band spray (after singling)	67,600	L.S.D. between treatment totals ($P = 0{\cdot}001$) = ± 3,500
10. Schradan spray at rosette stage	52,300	
11. Control (untreated)	81,200	

Statistical differences in aphid numbers between treatments were very high, although the degrees of control obtained with endrin and schradan were proportionately less than they were on 26 July. Alatae were still coming on to the lettuce after 26 July and the reduced control reflects a decline in insecticidal effectiveness with both materials. An apparent change in order between treatments 9 and 10 is evident in the aphid numbers recorded respectively on 26 July and 11 August. This shows that after the first sampling, the schradan treatment, probably because of the later application time, was more effective than was the endrin band spray.

1956 EXPERIMENT

Endrin, having given a high degree of control of *P. bursarius* in 1955, was used exclusively in the 1956 field experiment. The treatments are shown in Table 3. The seed dressings and band sprays were applied in the same way as in 1955 and the dust (50% concentrate diluted to 1·0% with talc) was applied as was the lindane dust in that year. The sprays were dilutions in water of a proprietary emulsifiable solution containing 19·7% (w/v) of endrin. Each treatment was replicated three times using a randomized block layout. The plot size and spacing of the plants after singling were as in 1955.

Spring migrants of *P. bursarius* were first seen on the lettuce on 30 June and a month later when the earliest plants had formed hearts, eight root systems in each plot were examined for aphids. These root systems were forked up and the aphid numbers determined. Wet weather had made the soil extremely friable and each root clump broke to the touch along small lines of fracture in which the aphids were hidden. The aphids, with their waxy secretions, were thus clearly visible against the dark soil and their numbers could be readily assessed in terms of geometric-progression grades as used by Wright & Wheatley (1953).

The aphid numbers found on 30 July are given in Table 3. The aphid populations on all plots were considerably lower than in the 1955 experiment, and in contrast to the results obtained in that year, endrin seed dressing alone gave little control. However, when this dressing was used in combination with a seedling spray (treatment 2) a marked reduction in population resulted. Two sprays of endrin to the seedlings also gave a high level of control and were more effective than one spray. All other treatments were comparatively ineffective.

A second series of samples taken on 23 August confirmed that aphid numbers were much lower on treatments 2, 6 and 7 than they were on any other plots.

TABLE 3. *Treatments and the resultant populations of* Pemphigus bursarius 1956

Treatment no.	Insecticide (endrin) Formulation	Concentration (%)	Application rate	Aphid nos. on roots of 24 plants 30 July
1	Seed dressing	50	100% of wt. of seed	495
2	As 1 + spray	50	As 1	
		0·1	8 fl. oz./40 row ft. A.S.	50
3	As 1 + spray	50	As 1	
		0·1	8 fl. oz./40 row ft. R.S.	310
4	As 1 + dust	50	As 1	
		1·0	1 oz./40 row ft. A.S.	210
5	As 1 + dust	50	As 1	
		1·0	1 oz./40 row ft. R.S.	190
6	Spray	0·2	8 fl. oz./40 row ft. A.S.	117
7	Spray	0·1	8 fl. oz./40 row ft. A.S.	
	Spray	0·1	8 fl. oz./40 row ft. R.S.	61
8	Dust	1·0	1 oz./40 row ft. A.S.	
	Dust	1·0	1 oz./40 row ft. R.S.	615
9	Control (untreated)	—	—	557

A.S.—after singling (3–4 true leaves); R.S.—rosette stage (12 true leaves).

1957 EXPERIMENT

In the 1957 field experiment, thimet 44D seed dressing was used for comparison with various endrin treatments; a preliminary germination test having shown that 10% by weight of thimet dressing to lettuce seed gave negligible phytotoxicity. The experimental layout was similar to that of previous years and seed dressings and spray were applied as before. Table 5 lists the treatments.

The sprays used after singling were applied on 20 June at a slightly later stage of plant growth than in the two previous years. The first spring migrants of *P. bursarius* were found on the lettuce on this date, but they did not become generally distributed over the crop until the end of June. The endrin emulsion in treatment 9 was applied to the soil around the base of each plant, the lower leaves having been moved aside.

Soil conditions permitted the same method of sampling as in 1956 to be used

on 30 July, when the first lettuces were ready for cutting. The aphid population figures so obtained are given in Table 4.

As in 1956 the aphid populations were low, but on all treatments except 2 and 9, they were significantly less than on the untreated plots. The best control appears to have been given by endrin seed dressing followed by endrin spray (treatment 3), a result confirming that obtained in 1956. It seems also that where a supplementary spray was used the amount of endrin seed dressing could be halved without markedly affecting the control obtained (treatment 4). The performance of the thimet 44D seed dressing was also enhanced by this additional spray.

TABLE 4. *Treatments and the resultant populations of* Pemphigus bursarius 1957

Treatment no.	Insecticide and formulation	Concentration (%)	Application rate	Aphid nos. on roots of 24 plants 30 July
1	Endrin seed dressing	50	100% of wt. of seed	150
2	Thimet 44D seed dressing	44	10% of wt. of seed	187
3	As 1 and endrin spray	50 0·2	100% of wt. of seed 8 fl. oz./40 row ft. A.S.	2
4	As 1 and endrin spray	50 0·2	50% of wt. of seed 8 fl. oz./40 row ft. A.S.	30
5	As 2 and endrin spray	44 0·2	10% of wt. of seed 8 fl. oz./40 row ft. A.S.	16
6	Endrin spray	0·2 0·2	8 fl. oz./40 row ft. A.S. 8 fl. oz./40 row ft. 2 wk. A.S.	52
7	Endrin spray	0·4	8 fl. oz./40 row ft. A.S.	101
8	Endrin spray	0·4	8 fl. oz./40 row ft. 2 wk. A.S.	88
9	Endrin emulsion	0·1	$\frac{1}{8}$ pt./plant to plant base 2 wk. A.S.	284
10	Control (untreated)	—	—	1587

A.S.—After singling (four true leaves). L.S.D. between treatment totals ($P = 0{\cdot}05$) = $\pm 1{,}432$.

Although the endrin spray treatment 8 and 'spot' treatment 9 were applied on the same day, the latter failed to give a significant level of control. The application of endrin to the soil immediately around the base of the plant in this treatment gave no foliage cover of insecticide and the outward penetration of the endrin through the soil was probably insufficient to prevent aphid colonization of the side roots.

Another series of samples of the root systems was taken at the end of August, but because predators had upset the relative aphid populations between plots, no further information on the performance of the treatments was obtained.

1958 EXPERIMENT

Diazinon was used for comparison with endrin in 1958. The treatments (Table 5) in each of four randomized blocks included seed dressings and band sprays as in the previous years, and soil treatments applied before the lettuce was sown. With the soil treatments the insecticides were dusted or sprayed evenly over the soil surface at rates equivalent to 2 and 4 lb./acre; the plots were then rotavated twice to a depth of 3 in.

TABLE 5. *Treatments and the resultant populations of* Pemphigus bursarius 1958

Treatment no.	Insecticide	Concentration (%)	Application rate	Aphid nos. on roots of 32 plants 31 July
1	Endrin seed dressing	50·0	100 % of wt. of seed	370
2	As 1 and endrin spray	50·0 0·2	As 1 8 fl. oz./40 row ft. A.S.	15
3	As 1 and endrin spray	50·0 0·2	50 % of wt. of seed 8 fl. oz./40 row ft. A.S.	4
4	As 1 and diazinon spray	50·0 0·2	50 % of wt. of seed 8 fl. oz./40 row ft. A.S.	11
5	Diazinon seed dressing	20·0	10 % of wt. of seed	424
6	As 5 and endrin spray	20·0 0·2	As 5 8 fl. oz./40 row ft. A.S.	47
7	As 5 and diazinon spray	20·0 0·2	As 5 8 fl. oz./40 row ft. A.S.	110
8	Endrin spray	0·1 0·1	8 fl. oz./40 row ft. A.S. R.S.	98
9	Endrin spray diazinon spray	0·1 0·1	8 fl. oz./40 row ft. A.S. R.S.	197
10	Diazinon spray	0·1 0·1	8 fl. oz./40 row ft. A.S. R.S.	14
11	Endrin spray	0·2	$4\frac{1}{3}$ fl. oz./sq.yd. B.So. (=2 lb. a.i./acre)	40
12	As 11	0·4	$4\frac{1}{3}$ fl. oz./sq.yd. B.So. (=4 lb. a.i./acre)	11
13	Diazinon spray	0·2	$4\frac{1}{3}$ fl. oz./sq.yd B.So. (=2 lb. a.i./acre)	49
14	As 13	0·4	$4\frac{1}{3}$ fl. oz./sq.yd. B.So. (=4 lb. a.i./acre)	9
15	Endrin dust	1·0	$\frac{2}{3}$ oz./sq.yd B.So. (=2 lb. a.i./acre)	95
16	Endrin dust	1·0	$1\frac{1}{3}$ oz./sq.yd. B.So. (=4 lb. a.i./acre)	4
17	Nil (control)	—	—	436

B.So.—Before sowing; A.S.—after singling (four true leaves); R.S.—rosette stage (twelve true leaves); a.i.—actual insecticide. L.S.D. between treatment totals—(P = 0·05) = ±403.

The first spring migrants were found on the lettuce on 23 June when the 'after-singling' band sprays were applied and on 7 July when the second sprays were applied at rosette stage, a few subterranean colonies of *P. bursarius* were just forming.

On 31 July the plots were sampled and the aphid population figures, shown in Table 5, were obtained using the field method of assessment described earlier.

Seed dressing with endrin failed to control *P. bursarius* but when supplemented by a seedling spray with endrin or diazinon a good control of the pest resulted. Diazinon seed dressing gave no control. When worked into the soil before sowing both endrin and diazinon considerably reduced aphid numbers, particularly at the higher rate of application. Two diazinon sprays also gave a good control.

Discussion and conclusions

It must first be emphasized that this paper simply records the effects of endrin against lettuce root aphid under the conditions described, but cannot serve at present as the basis for practical recommendations because the levels and persistence of the endrin residues have not been determined.

As a seed dressing, endrin gave a high degree of control of lettuce root aphid in 1955 and 1957 but not in 1956 and 1958. Other seed dressings (thimet 44D and diazinon) gave inadequate control. However, a consistently good control was obtained when a seed dressing with endrin was followed by an endrin or diazinon spray applied along the rows just after the lettuce had been singled.

The addition to seed of its own weight of endrin 50% dust induced no phytotoxicity when freshly dressed seed was sown, but it was found that germination became impaired if the dressed seed was stored before being sown. This impairment was reduced when the weight of dressing was halved and, because no additional control resulted from the heavier dosage (see Table 5) the lower dosage is to be preferred. If a supplementary spray is used, it is applied to the lettuce 5–6 weeks before harvesting begins. During this time the lettuce grows from small seedlings showing their fourth true leaf to fully hearted plants of about sixty leaves. Amounts of endrin residue on the harvested crop are therefore likely to be very small, particularly when the senescent, earliest-formed leaves are removed before marketing.

In the absence of seed dressing, a good control of the aphid was obtained with two spray applications of endrin or diazinon, one applied after singling and the other at the early rosette stage. Control measures involving two foliage sprays of endrin may, however, prove to be unacceptable because of the residues. Diazinon is safer to use than endrin and future work may show that adequate control is obtainable with a spray concentration lower than 0·1% diazinon.

Endrin or diazinon at 4 lb./acre, worked into the top 3 in. of soil before the crop was sown, controlled the aphid as effectively as any of the above methods. Such soil treatments overcome the problem of spray residues on the foliage and appear to be a much more acceptable means of controlling *P. bursarius*.

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THE TOXICITY OF SOME INSECTICIDES TO FIFTH-INSTAR NYMPHS OF THE DESERT LOCUST

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(With 3 Text-figures)

The toxicities of the insecticides γ-BHC, diazinon, dieldrin, DNC, heptachlor and parathion to fifth-instar hoppers of the desert locust were determined. Parathion and dieldrin were the most toxic, followed in descending order by heptachlor, γ-BHC, diazinon and DNC. The resistance of the hoppers to γ-BHC and DNC changed appreciably with age within the instar.

The effects of some factors which might influence the resistance of the insects were examined and are discussed.

Introduction

Apart from the work of Pearson (1960) with dieldrin, no work has been published in which the absolute toxicities of insecticides to grasshopper or locust nymphs of exactly known ages have been determined. Mostly adults have been used (Weinman & Decker, 1949; MacCuaig, 1958) but some workers (Brown & Hurtig, 1947) have used mixtures of nymphs and adults. When a single nymphal instar has been used exact dosages have not been determined (Pradhan & Bindra, 1956), whilst in other experiments neither the instar nor the dose applied (Smit, 1951) has been known exactly, so that only general comparisons could be made.

Age and physiological state within the stadium are known to affect resistance to insecticides. Using *Drosophila melanogaster*, Kerr (1954) found that resistance of male flies decreased with age up to 5 days and then increased up to 9 days, whereas the resistance of females increased rapidly during the first 3 days and thereafter changed little. With adult mosquitos the stage in the feeding cycle at which the insect is dosed affects resistance (Hadaway & Barlow, 1956), whilst against larvae of *Aedes aegypti* the concentration required to kill 50% of the larvae after a given exposure time varies by a factor of over 100 in 6 days (Parker, 1957).

The experiments described below were therefore designed to determine the resistance of fifth-instar hoppers (nymphs) of the desert locust *Schistocerca gregaria* (Forsk.) to several insecticides at various ages after moulting. Six insecticides were used and applied to hoppers at five ages during the fifth instar. It was found that the variation between batches was considerable and some attention was given to factors which might account for the differences between apparently identical experiments. The insecticides used were chosen because they might be suitable, or are actually used, for practical control operations against hoppers, or because they have been used against grasshoppers or adult locusts. They are all insecticides for which the toxicities against adult locusts are known.

Materials and methods

The locusts were derived from stocks bred in the laboratory of the Anti-Locust Research Centre, London. The conditions under which they were reared were similar to those described by Hunter-Jones (1956). Within 16 hr., at most, after the fourth moult the hoppers were generally transferred to metal and celluloid cages ('larval cylinders'; approximately 11 l. capacity) in groups of up to thirty and kept until they reached the age required for the experiment. Approximately fifty insects, the contents of two or three larval cylinders, were used to obtain one LD 50 and one LD 99 at each age. The insecticide applied and the age at which each group was used were decided by a semi-random process. The main influence affecting randomization was avoidance of dosing at week-ends. (When the age of the hoppers is not stated they were dosed 4–7 days after the fourth moult.)

The insecticides used were γ-BHC, diazinon, dieldrin, DNC, heptachlor and parathion, dissolved in or diluted with kerosene extract bottoms (KEB) so that the solutions were roughly equitoxic, with concentrations ranging from 1 to 20%. The solutions were applied by means of the micro-drop syringe to the intersegmental region between the first and second abdominal sternites. Each insect was weighed before dosing and, generally, the quantity of insecticide applied was adjusted to give a preselected amount of ingredient per gramme body weight. Food was available to the insects before dosing, but they were not fed again until about 24 hr. after treatment. In each of the experiments in which the technique was varied, control groups were treated in the manner just described and obtained by randomization from within the same batch. The insects were recorded as dead if they could not right themselves when turned upside down 24 hr. or more after dosing. Locusts affected to this extent were never seen to recover. Most of the toxicity data were obtained using the methods given by Finney (1952), but graphical estimations only from probit regression lines were made.

Results

Relation between resistance and age after moulting

The principal experiments were those designed to study the resistance in relation to age within the instar, and the results are shown in Table 1. The wide range in LD 50 can be seen, at least twofold for each insecticide, excluding parathion with which relatively few insects were dosed. Within each experiment with one insecticide the probit regression lines were drawn parallel. On no occasion was significant heterogeneity introduced by this procedure.

On examining the γ-BHC results it will be seen that the LD 50 at 2·5 and 4·5 days is greater than at other ages, the average at these ages (11·05 μg./g.) being significantly greater than those at other ages. To diazinon the hoppers appeared to be slightly more susceptible at 8·5 days than at other ages whilst with DNC there is a striking increase in resistance with age, hoppers more than 5 days old within the instar being about twice as resistant as those 1–3 days old. With the other insecticides no significant change in resistance with age could be detected.

Relative resistance of the sexes

With γ-BHC, diazinon and heptachlor, large numbers of hoppers were dosed at 4·5 days, so that the resistance of each sex could be determined separately. The results of these experiments are shown in Table 2. The doses were applied per

TABLE 1. *Relation between resistance and age of hoppers of the desert locust after moulting*

Insecticide	Exp. no.	No. of insects	Age of hoppers (days)					Slope of common regression line
			0·5–1·3	*2–3*	*4–5*	*6–7*	*8–9*	
			LD 50 (μg./g.)					
γ-BHC	1	305	8·1	10·8	10·3	5·3	5·3	6·96 ± 0·74
	2	407	8·2	13·3	9·8	4·8	7·6	5·37 ± 0·68
Diazinon	1	338	13·0	14·5	13·0	13·3	6·6	6·16 ± 0·70
	3	359	11·9	8·6	18·9	10·0	8·5	4·67 ± 0·51
Heptachlor	1	321	5·6	5·4	7·3	6·3	3·7	5·93 ± 0·86
	2	246	6·1	7·1	5·6	5·3	4·6	6·39 ± 0·79
Parathion	3	165	1·5	> 2·0	1·6	1·5	1·6	6·65 ± 1·04
			0·3–1·0	*1·3–3*	*3–5*	*6–8*	*8–9*	
Dieldrin	2	255	2·9	2·0	2·3	1·7	2·2	5·03 ± 0·67
	4	258	1·3	1·4	1·2	1·7	—	6·9
	5	108	—	—	2·5	2·0	—	5·0
			0·3–1·3	*1·3–3·9*	*3–5·9*	*4·1–6·7*	*6–7*	*8–9*
DNC	3	323	18·6	21·3	24·2	—	38·8	43·1	6·98 ± 0·69
	6	117	12·6	—	29	—	—	—	10·5
	7	107	12·5	> 20	—	—	< 28	—	—
	8	150	—	15	> 20	34	—	—	9·3

TABLE 2. *The relative susceptibility to insecticides of male and female hoppers of the desert locust*

Insecticide	Age in 5th instar when dosed (days)	Sex	No. of insects	LD 50 (μg.) per locust	LD 50 (μg.) per unit body weight	Relative susceptibility of males/g.
γ-BHC	4·5	♂	148	12·1*	9·3	1·17
		♀	106	16·6	10·9	
Diazinon	4·5	♂	49	20·3*	13·9	0·99
		♀	60	25·0	12·9	
Dieldrin	0·3–8·0	♂	113	—	1·2	1·08
		♀	154	—	1·3	
DNC	0·8	♂	41	14·7	18·7	0·99
		♀	43	17·2	18·5	
	6·0–9·0	♂	73	53·4*	38·7	1·07
		♀	47	73·6	41·6	
Heptachlor	4·5	♂	63	8·1*	6·8	1·15
		♀	51	11·1	7·8	
Parathion	0·3–9·0	♂	60	—	1·5	1·00
		♀	81	—	1·5	

* Differences significant at the 5 % level.

locust regardless of weight, although the weights were recorded enabling the average doses applied in μg./g. to be determined. For these insecticides the resistance of the female hoppers is significantly greater than that of the males when the dose is measured per locust, but not when the weight of the insects is taken into account.

For dieldrin and parathion, doses per locust are not relevant because hoppers of mixed ages within the fifth instar were used, but for these insecticides too there is no difference between the sexes when the doses are measured per unit body weight.

For DNC, taking into account the variation of resistance with age, locusts approximately 1-day-old within the fifth instar were used, dosed per locust regardless of weight. Because of the small number of insects used the greater resistance of the females was not statistically significant. However, when the 6·5 and 8·5 day-old fifth instars (DNC Exp. 3, Table 1) were separated into the sexes a significant difference was found between them when the average dose per locust was calculated.

Resistance and body weight

Owing to the variation in resistance between groups, it was impossible to carry out analyses from the data shown in Table 1 using the probit plane technique (Finney, 1952). An alternative method was therefore designed minimizing the differences between groups. Each group was divided into light and heavy insects and the ratio of weights calculated, treating males and females separately. For each experiment (except DNC) all the heavy insects from each group were combined regardless of age or sex, as were the light insects. The mean ratio of the weights was calculated together with the LD 50's and fiducial limits of their ratios (Table 3). An LD 50 ratio of unity indicates that the resistance of the two groups, to doses measured in μg./g., is the same, that is, that resistance is proportional to body weight. LD 50 ratios having the same value as the weight ratios would indicate that the resistance was independent of weight.

For individual insecticides it is probably unsafe to draw definite conclusions from this analysis because, for example, the two experiments with γ-BHC yielded opposite results. Considered as a whole, however, the ratios are nearer unity than 1·22, the mean weight ratio. In fact, the mean weighted ratio of the LD 50's of light : heavy insects for all the experiments was 0·924 with 95 % confidence limits 0·888–0·950, significantly less than unity. This indicates that the heavier hoppers, although given more insecticide in proportion to their weight, require quantities greater than this to yield the same kill as lighter ones.

Resistance of hoppers deprived of food

The hoppers were normally not fed within 24 hr. after dosing. With slow-acting insecticides (dieldrin and heptachlor), symptoms frequently did not appear for 2–3 days after dosing, whilst with quick-acting insecticides (diazinon and DNC), action was usually complete within 24 hr. In experiments with these quicker-acting insecticides a period of starvation increased susceptibility, but the effect of

not giving the insects food after dosing was small compared with the effect of removal of food 24 hr. before dosing (see Table 4).

In other experiments a comparison was made between insects supplied with abundant grass before dosing and others deprived of food and generally water, for

TABLE 3. *The relative susceptibility to insecticides of different weight groups of the desert locust*

Insecticide	LD 50 of light group (μg./g.)	LD 50 of heavy group (μg./g.)	Ratio of LD 50's light : heavy, and 95 % fiducial limits of the ratio	Ratio of weights
γ-BHC (*a*)	7·83	5·69	1·38 0·90–2·10	1·22
(*b*)	5·36	7·01	0·76 0·51–1·15	1·20
Diazinon (*a*)	11·5	11·4	1·00 0·83–1·19	1·20
(*b*)	11·2	10·5	1·06 0·83–1·36	1·22
Dieldrin	1·89	2·17	0·87 0·50–1·38	1·24
Heptachlor (*a*)	12·8	15·6	0·82 0·61–1·38	1·21
(*b*)	4·99	5·73	0·87 0·75–1·01	1·24
Parathion	1·54	1·78	0·86 0·73–1·02	1·21
	Weighted mean		0·924 0·888–0·950	1·22

TABLE 4. *Effect of removal of food on resistance of fifth-instar hoppers to diazinon* (LD 50's in μg./g. *and standard error*)

Treatment	Abundant food available before dosing	No food available for 24 hr. before dosing
Abundant food available after dosing	20·7 (2·6)	12·8 (1·3)
No food available after dosing	19·7 (2·2)	11·3 (1·2)

Table 5. *Effect of lack of food, for 24 hr. before dosing, on resistance*

Insecticide	Total no. of insects used	LD 50 of control group	LD 50 of starved group	Relative susceptibility of starved group
γ-BHC	141	> 14	> 14	—
Diazinon	130	10	5·2	1·9*
Dieldrin	96	2·6	2·9	0·9
DNC 1–2 days	70	15·1	7·8	1·9†
5–7 days	80	31·0	15·4	2·0†
Heptachlor	96	9·4	11·7	0·8
Parathion	135	1·86	1·55	1·2

* Ratio significantly different from unity at the 5 % level.
† Ratio significantly different from unity at the 1 % level.

24 hr. before dosing. Neither group was fed for 24 hr. after dosing. The results are shown in Table 5. With dieldrin, heptachlor and parathion, lack of food before dosing had no significant effect on resistance. Using γ-BHC, 14 μg./g. was the maximum dose given, and five out of eighteen died from this dose in the group fed before dosing and six out of eighteen in the group starved before dosing; clearly there was little difference between the two treatments with this insecticide. The results using DNC and diazinon, as already stated, show that depriving the insects of food for 24 hr. before applying the insecticide increased their susceptibility to these poisons.

Resistance to insecticides injected into the body cavity

Dilute solutions of the insecticides were prepared in propylene glycol which had previously been found to be a suitable solvent and non-toxic in the quantities injected. Each injection was made using a fine hypodermic needle passed forwards and upwards through the soft cuticle dorsal to the hind coxa so that the tip lay between the gut and the body wall.

For each insecticide there was little change in resistance when the insecticide was injected instead of being applied superficially, and in no case was the change statistically significant (Table 6). Results with dieldrin (unpublished) similarly showed no significant difference in the toxicity by injection compared with contact.

Although no exact comparison between contact and injection toxicity was made with DNC, the results are particularly interesting because they demonstrate that the increased resistance to contact action shown by older hoppers is also shown when the insecticide is applied by injection although the change appears to be smaller.

Table 6. *Resistance to the insecticides applied by injection*

Insecticide	Total no. of insects used	LD 50 (μg./g.) by Injection	LD 50 (μg./g.) by Topical application	Relative toxicity by injection
γ-BHC	80	7·9	8·2	1·03
Diazinon	80	12·5	16·8	1·34
DNC	161	13·6 (1–2 day)	—	—
		17·8 (7–9 day)	—	—
Heptachlor	92	3·5	3·5	1·00
Parathion	120	2·7	2·4	0·89

In each case the action appeared to be more rapid when the insecticide was injected than when it was applied to the surface of the abdomen. Thus, by injection all the deaths from DNC occurred with 2·5 hr., whereas by topical application only eleven out of twenty-five deaths occurred in 5 hr. With γ-BHC virtually all the deaths took place within 7 hr. after injection, whereas very few deaths occurred within 6 hr. by topical application. Similarly with dieldrin, nearly 65% of the total number of deaths took place within 3 days from injection, whereas only about 30% of the total had occurred from topical application.

The effect of the solvent on toxicity

The toxicities of the insecticides dissolved in xylene were compared with those in KEB. Only γ-BHC and diazinon were found to be as toxic in xylene as in KEB.

In other experiments with dieldrin, the toxicity of dieldrin in a xylene emulsion in water was found to be only about one-quarter as toxic as that of dieldrin in oil (MacCuaig, 1956*b*).

TABLE 7. *The effect of the solvent on toxicity of the insecticide*

Insecticide	Total no. of insects used	LD 50 with KEB (μg./g.)	LD 50 with xylene (μg./g.)	Relative toxicity in xylene
γ-BHC	80	8·2	9·0	0·91
Diazinon	80	16·8	20·2	0·83
Dieldrin	96	2·6	5·3	0·49**
DNC	71	15·1	>25	<0·60**
Heptachlor	80	3·5	6·3	0·56**
Parathion	160	2·4	4·2	0·57*

* Denotes significant difference at the 5 % level.
** Denotes significant difference at the 1 % level.

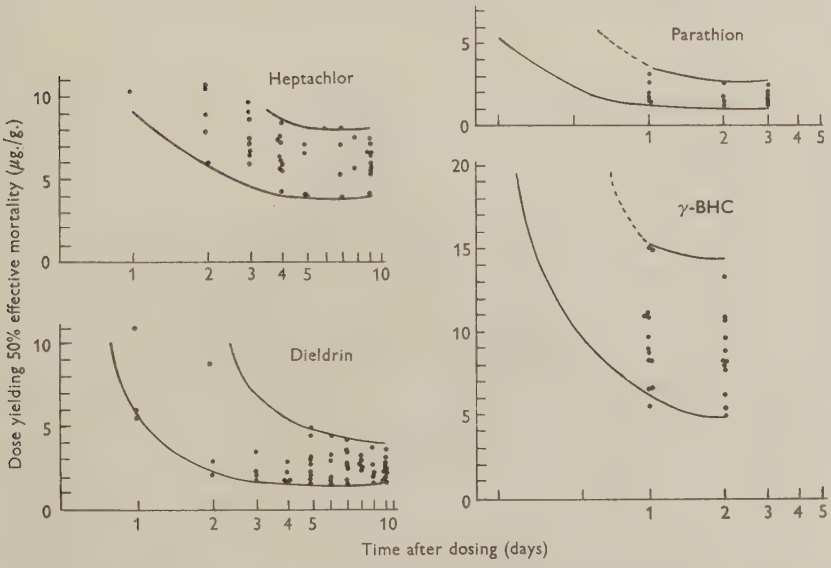

Fig. 1. Relative toxicity and speed of action of some insecticides to fifth-instar *Schistocerca*.

Contact toxicity: summary of results

The results of all suitable experiments (Tables 1 and 4–7) may be conveniently summarized by determining the means of the LD 50's and LD 99's and their standard deviations (using in all cases log. values). To do this, all the experiments

in which the insecticides were applied in oil solution to insects not deprived of food for 24 hr. before dosing were used (excluding DNC). This summary of the results is shown in Table 8. For DNC a range only is given because the change in

Table 8. *The contact toxicity of the insecticides used applied in oil solutions*

Insecticide	Numbers: Insects	Numbers: Determinations	LD 50 (μg./g.)	LD 99 (μg./g.)
γ-BHC	844	14	8·7 4·4–16·8*	19·5 9·6–40*
Diazinon	802	14	12·7 6·5–25*	33·7 15·7–72
Dieldrin	865	15	1·9 1·0–3·5*	4·6 2·3–9·1*
DNC	707	—	12·5–43†	20–90†
Heptachlor	655	13	5·7 3·3–10·0*	14·3 6·6–31*
Parathion	315	8	1·9 0·9–4·0*	3·6 2·2–6·6*

* 95 % fiducial limits of the results from the series of experiments.
† Range only.

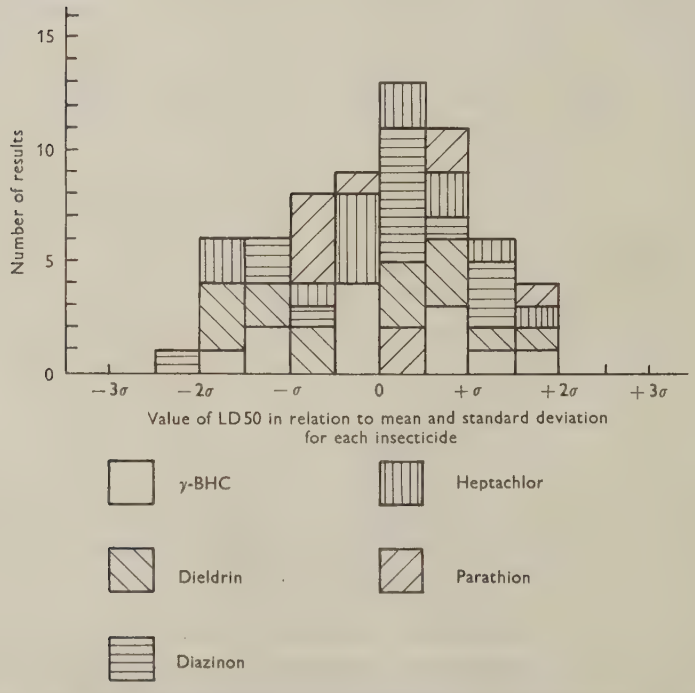

Fig. 2. Distribution of the LD 50 about their means for the combined results shown in Table VIII.

resistance with age is so great. With γ-BHC part of the range may be attributed to an increased resistance at 2·5–4·5 days, whilst with diazinon part may be attributed to a decreased resistance at 8·5 days. However, the range of results for these two insecticides is about the same as for the insecticides with which no change in resistance with age has been found.

The speed of action of the insecticides is shown in Fig. 1, where the doses yielding a 50% kill in a given number of days are shown for γ-BHC, dieldrin, heptachlor and parathion. The action of DNC was complete in 0·5–1·0 days and that of diazinon in about 1 day; the cyclodiene insecticides, dieldrin and heptachlor, are the slowest in action.

For adult locusts dosed with DNC, MacCuaig (1958) plotted a frequency histogram of the LD 50's and LD 99's and found that (on a log. scale) they did not differ significantly from a normal distribution. A similar procedure was followed here using the results from Table 8, by combining the results of each insecticide (again excluding DNC). Using the means and standard deviations already obtained, the number of LD 50's found in each half standard deviation from the mean was determined and plotted on a composite histogram (Fig. 2). Plotted in this way these results do not differ significantly from a normal distribution ($\chi^2(9) = 7{\cdot}10$).

Discussion

Many experiments have been carried out to compare the contact toxicity of groups of locusts obtained from the same batches by a randomization procedure and treated with the same insecticide. The standard deviation of the differences between the LD 50's (on $\log_{10}$ scale) was 0·0263 ($1 \pm 0{\cdot}18$). This means that differences between LD 50's of $\times$ 1·12 may be regarded as the minimum which can be detected reliably by the combination of insects, methods and apparatus used in these experiments. The observed variability was much greater than this and cannot therefore be attributed entirely to the methods used but must be sought in the insects themselves.

Cuticle structure has long been regarded as a factor of considerable importance in affecting the toxicity of an insecticide (see, for example, Brown, 1951). With nymphal *Schistocerca*, Pradhan & Bindra (1956) concluded that both the percentage of wax in the cuticle and the thickness of the cuticle were important factors affecting resistance between instars. Yet in the experiments described here the toxicity by injection was similar to that by contact, provided that oil solutions were used. When DNC was injected, the resistance of the insects increased with age in the same way as when the insecticide was applied by contact. With xylene as the solvent, the cuticle sometimes acted as a barrier and sometimes did not. Thus the effect of the cuticle varies with the solvent used and it is possible that with some solvents slight changes in cuticle structure may appreciably affect toxicity. It may be significant that Pradhan & Bindra used γ-BHC in a carrier consisting of 95% water.

Way (1954) using *Diataraxia oleracea* has considered the effect of weight on resistance. With parathion he found that resistance was proportional to weight but with γ-BHC and DDT it was not. However, since the most important factor

affecting weight was age, the negative conclusion shows primarily that resistance changes within the instar. It is necessary to distinguish three separate aspects of weight changes: first, changes of weight between age groups; secondly, variation in weight between different groups of the same age; and thirdly, weight variation within single groups of the same age. These are not necessarily related. Thus, with DNC applied to adult *Schistocerca*, resistance was proportional to weight within groups of the same age and between groups of different ages (MacCuaig, 1956*a*), but not between different batches of the same age but of different weights (MacCuaig, 1958).

A fourth factor, that of weight differences between the sexes, is sometimes important, but as is shown by the results in Table 2, it can be ignored in these experiments.

The average weights of the insects at the main ages are shown in Table 9. For heptachlor, parathion and dieldrin resistance per locust is clearly proportional to weight change between ages. Resistance to DNC is not related in this manner; resistance to diazinon is related to weight up to 6·5 days, but at 8·5 days, although the insects are nearly the same weight, their resistance has decreased. With γ-BHC the pattern appears to be still more complicated (see Table 1).

Table 9. *Average weights of* Schistocerca gregaria *hoppers* (*g.*)

	Mean age and range from time of moult (days)				
Sex	0·8 ± 0·5	2·5 ± 0·5	4·5 ± 0·5	6·5 ± 0·5	8·5 ± 0·5
Males	0·81	1·05	1·25	1·52	1·55
Females	0·96	1·25	1·56	1·84	1·95

Where possible, groups of hoppers of the same age were examined to see whether their weight could be related to their resistance. There was no striking correlation, but the data were probably too fragmentary to enable small trends to be detected.

Seasonal weight changes may occur between groups of hoppers of the same age. To examine this possibility the average weight of each group of hoppers in the larval cylinders was obtained during March, April and October, 1957. At each age the variation within months was greater than that between months (Fig. 3). The hoppers weighed and dosed during March and April were subjected to different feeding régimes. Those weighed in April were fed with abundant fresh grass, whereas those weighed in March and reared during February and March, had to be fed mainly on kale and bran or fish meal. Way (1949) has shown that changes of food may affect toxicity through altering the weight of the insects, whilst Lofgren & Cutkomp (1956) found that high protein diets, for example, fed to the American cockroach yielded the most susceptible insects. These factors again did not operate in the present experiments, although feeding certainly can affect the resistance of fifth-instar *Schistocerca* to DNC. One group of hoppers showed no increased resistance when dosed 3–5 days after the fourth moult and their average weight was no greater than that of hoppers one day after moulting (MacCuaig, 1956*b*).

The evidence available on the relation between resistance and weight within

groups has already been given. It can be expressed simply in a notation similar to that used by Busvine (1957):

$$(\text{resistance}) \propto (\text{weight})^h.$$

In the analysis shown in Table 3, h would be zero if the LD 50 ratio of light : heavy groups was the same as the ratio of weights, whilst it would be unity if the LD 50 ratio was unity. The mean result, however, yielded a value of $h = 1{\cdot}4$.

The effects of the holding temperature after treatment were not studied in these experiments because it was not believed that they would be large relative to those of other factors. Using DNC against adult *Schistocerca*, identical kills were obtained at 25 and 36° C. (MacCuaig, 1956*a*), whereas with γ-BHC applied to hoppers of the same species no difference in kill was obtained between temperatures of 19·5 and 38·5° C. (MacCuaig, 1955). Even where temperature does affect toxicity, the differences are usually small. Thus Potter & Gillham (1946), using DNC against

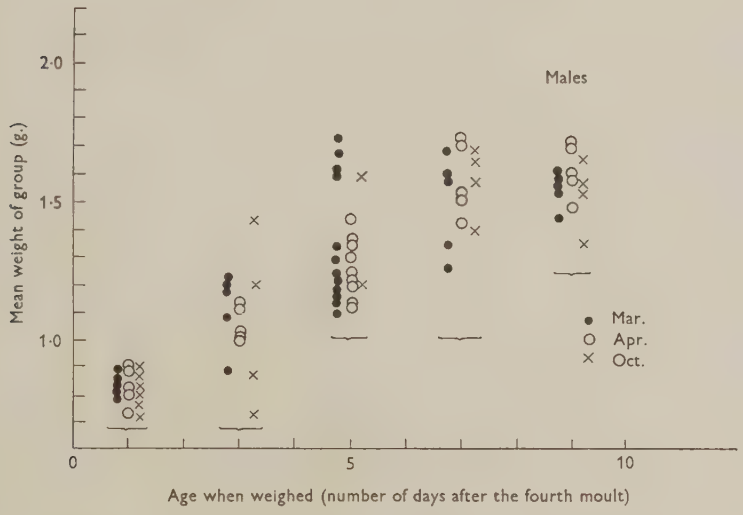

Fig. 3. Average weights of the males in each group of fifth-instar hoppers used during March, April and October, 1957.

Tribolium, found that the potency of the insecticide increased by 46% when the temperature fell from 27 to 16° C. Similarly with γ-BHC, dieldrin and diazinon, Hadaway & Barlow (1957) found that the resistance of the housefly at 20° C. was, at the most, 55% more than at 30° C.

After a short period of starvation, hoppers become more active (Ellis, 1951) and the composition of the body fluids change (Hoyle, 1954), so that it is not surprising that starvation can affect resistance (to DNC and diazinon). With the slower acting insecticides (γ-BHC, dieldrin and heptachlor) a change in resistance may be masked because the insects are fed again before the onset of the irreversible processes

leading to death. Parathion would thus be expected to be intermediate in its action, and indeed the resistance of the starved group (p. 327) is less than that of the control although the difference is not significant.

The effect of applying the insecticides dissolved in xylene is in all cases to reduce the toxicity, but the reduction is small and non-significant for γ-BHC and diazinon. Xylene rapidly evaporates and leaves the pure insecticide deposited on the insect. Solid insecticides such as DNC and dieldrin might be expected to be less effective as a result of this action, yet γ-BHC is a solid whilst parathion, which is a liquid, appears to be significantly less toxic in xylene than in oil solution. Pearson (1960), in determining the contact toxicity of dieldrin, used a mixture of xylene and acetone as a solvent, and obtained LD 50's of 5·2–8·0 μg./g. for fifth-instar *Schistocerca* hoppers. These results are clearly consistent with the one recorded here for dieldrin dissolved in xylene.

The data presented in Table 8 are adequate, for practical purposes, for determination of the relative potencies, although no single figure can be given as an estimate of the relative potency of some insecticides. It will be seen that parathion and dieldrin are by far the most toxic, with heptachlor about one-third as toxic, and γ-BHC and diazinon less than one-quarter, whilst DNC may be less than one-twentieth as toxic for the older groups.

Expressed as LD 50's in μg. active ingredient/g. locust body weight, only γ-BHC and parathion are similar in toxicity to both adults and fifth-instar hoppers. Heptachlor is about twice, and dieldrin about three times, as toxic to hoppers as to adults. Diazinon, on the other hand, is less than half as toxic to hoppers as it is to adults; indeed, using the values for adults given by MacCuaig (1958), fifth-instar hoppers 4–7 days after moulting are more resistant than adults. The decrease in resistance at 8·5 days, just before the final moult, is perhaps associated with the decreased resistance of adults.

DNC is again exceptional, since it is known that to doses measured in μg./g., adults show no change in resistance with age from 38 hr. to 38 days after moulting (MacCauig, 1956*a*). As with diazinon, resistance to DNC measured per locust is at a maximum for late fifth-instar hoppers, the LD 50 reaching an average value of about 60 μg./locust at 8·5 days, whereas for adults it did not exceed 50 μg./locust.

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INSECTICIDAL CONTROL OF PAEDOGENETIC CECID LARVAE IN MUSHROOM BEDS

I. INCORPORATION OF INSECTICIDES INTO COMPOST

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(With 3 text-figures)

γ-BHC, incorporated at 50 p.p.m. in mushroom compost during the last turn of composting delayed the reproduction of paedogenetic cecid larvae without reducing the yield of mushrooms. Other insecticides were less satisfactory as they either did not control the larvae, were not sufficiently persistent or decreased the yield. Large cecid populations did not depress yields on normal composts but a proportion of the larvae migrated on to the sporophores, thus reducing the value of the crop.

Introduction

Mushroom beds are commonly infested by cecid larvae which may affect production either by attacking the mycelium, by swarming up on to the mushrooms themselves or by disease transmission. The swarming habit is especially serious in the United States of America where processers find great difficulty in eliminating larvae from canned mushrooms; and in this country larvae on the caps and gills may prevent marketing of affected produce.

In Britain there are several species recorded as pests (Hussey & Wyatt, 1958) but only three, *Heteropeza pygmaea* Winn., *Mycophila speyeri* Barnes and *M. barnesi* Edwards, are of frequent economic importance. All these species have the following typical life-history. Fertilized female flies lay eggs in the compost soon after the introduction of mushroom spawn and the resulting larvae reproduce paedogenetically and complete many generations before a pupal phase occurs. The development of pupae is preceded by 'pupa-larvae' characterized by the sternal spatula normally found in Cecidomyiid larvae but, in paedogenetic species, restricted to this prepupal phase. The development of this phase occurs almost simultaneously throughout the larval population and a mass adult flight usually follows.

Examination of compost samples from many commercial farms showed that most contained at least a few larvae despite the widespread use of insecticides as dusts, smokes or aerosols against the other mushroom pests, species of Sciaridae and Phoridae. Sometimes cecid infestations result from inefficient hygiene following earlier crops, but Hussey & Wyatt (1958) showed that efficient peak-heating should kill all cecid larvae.

Moreton (1955, 1958) recommended the use of BHC and malathion in the compost before the trays are filled, but was uncertain whether or not insecticides would retain their properties under 'peak-heat' conditions, during which compost is

pasteurized at temperatures between 130–140° F. with evolution of variable quantities of ammonia. As these doubts were also expressed by some growers, experiments were planned to study the subsequent insecticidal efficiency of materials incorporated into the compost at filling.

Earlier workers have tested some materials for phytotoxicity and taint, for instance, Moreton (1955) who stated that malathion applied at 89 p.p.m. would depress yield but not at 22 p.p.m. (1 lb./5%/ton). Moreton & John (1955) stated that aldrin at 17 p.p.m. would not affect mycelial growth and Moreton (1955) reported that γ-BHC at 10 p.p.m. would not cause the crop reductions associated with mixed isomers of BHC. Shanahan (1948) claimed that even at 24 p.p.m. γ-BHC did not inhibit mycelial growth, reduce cropping or cause tainting. Rasmussen (1956) was the only worker to test, by means of a replicated trial, the effect of insecticides applied to the surface of mushroom beds. He sprayed emulsions of aldrin, diazinon, BHC and parathion at rates up to eight times the normal strength and concluded that only the highest levels of BHC reduced yield. His published data are difficult to analyse, but there is apparently a tendency for yield reductions at the higher concentrations of all the insecticides used, although his figures for BHC do not differ significantly from the controls at any concentration.

Experimental

Incorporation in compost of insecticides as dusts*

Experiment 1

Method. Published data on the concentrations at which insecticides could be included in composts without affecting cropping ranged from 10 p.p.m. BHC (Moreton, 1958) to 67 p.p.m. diazinon (Wood, 1956). Larger concentrations were used in the first experiment so that any losses in crop due to phytotoxicity would be emphasized. The experiment was laid out to a randomized block design with four replications of the following eight treatments: control; DDT 500 and 100 p.p.m.; γ-BHC 500 and 100 p.p.m.; aldrin 500 and 100 p.p.m. and diazinon 100 p.p.m. Each plot (23 × 27 in.) contained 50 lb. of compost and the insecticides were applied as dusts, with 100 g. of kaolin as filler. Kaolin was also applied to the control plots. Mixing was done by shaking on the dust as the compost was turned before filling the trays. Immediately before casing, all the plots were inoculated with equal quantities of water extract from cecid-infected compost. Both *Heteropeza pygmaea* and *Mycophila speyeri* were introduced in this manner but, as the

* BHC (mixed isomers) 1,2,3,4,5,6-hexachloro *cyclo*hexane
Malathion *S*-1,2-di(ethoxycarbonyl)ethyl-dimethylphosphorothiolothionate
DDT 1,1,1-trichloro-2,2,bis(*p*-chlorophenyl)ethane
Diazinon diethyl-6-methyl-2-*iso*propyl-4-pyrimidinyl phosphorothionate
Rogor *O,O*-dimethyl-*S*-(*N*-methylcarbamoylmethyl)phosphorothiolothionate
Dieldrin 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4,5,8-dimethanonaphthalene
Aldrin 1,2,3,4,10,10-hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4,5,8-dimethanonaphthalene
Dipterex *O,O*-dimethyl-2,2,2-trichloro-1-hydroxethyl phosphonate
Dimetan 5,5-dimethyl-dihydroresorcinol dimethyl carbamate

latter did not establish well, all the subsequent population counts did not distinguish between the two species. At intervals after filling, samples were taken from each bed to a predetermined randomized design. Three cores (1·6 in. diam.) cut from each plot with a hollow, sharpened steel cylinder, were mixed, and 20 g. extracted in a Baermann funnel lined with Tygan mesh for 20 hr. A further 20 g. were retained for residue analysis, and were extracted in sealed Kilner jars, without preliminary drying, by shaking with benzene (DDT and diazinon) or chloroform (BHC). Micro-methods of analysis were employed as it was anticipated that residues might become considerably depleted during the experiment.

Results. At the relatively high concentrations used the insecticides considerably affected the yield and number of mushrooms (Table 1). The control plots consistently yielded more than any of the treatments. DDT at 100 p.p.m. reduced the

Table 1. *Total yields, numbers of mushrooms and cecid populations of beds treated with insecticidal dusts* (Exp. 1)

Insecticide	Control	DDT		BHC		Aldrin		Diazinon	Sign. Diff. 5%
Concentration in compost (p.p.m.)	0	500	100	500	100	500	100	100	—
Total yield (g.)	3725	1168	1957	1692	2986	857	2372	2960	1239
Total no. mushrooms	678	222	300	333	521	171	428	419	199
Mean wt./ mushroom (g.)	5·49	5·26	6·52	5·08	5·73	5·12	5·54	7·07	—
No. larvae per 20 g. (n) 7 weeks after spawning	435	200	6·3	1	6	1	8	200	—
$\Sigma \log (n+1)$	7·74	5·63	8·08	0·30	1·56	0·30	1·66	4·53	2·17

Table 2. *Insecticide residues (p.p.m. moist compost) at different periods after filling*

	DDT			BHC			Diazinon	
Amount added ...	0	100	500	0	100	500	0	100
Sample after								
10 days	—	82	398	5	42	253	6	20
5 weeks	9	120	627	10	58	112	1	10
12 weeks	3	118	547	1	16	90	1	2
19 weeks	10	100	713	1	20	138	0·3	2

crop by 47% and by as much as 69% if applied at 500 p.p.m., BHC at 500 p.p.m. decreased yield by 55% but at 100 p.p.m. the 20% reduction was not significant at the 5% level. Diazinon was of particular interest, as although the number of mushrooms fell by 38%, the yield was only reduced by 21% (not significant), and numerical losses were partially balanced by larger mushrooms. All the treatments followed a common trend in weekly crop yields, producing a major peak about the third week with a smaller peak in the sixth. The control plots had a third peak in the ninth week when the yield of all the treated plots was dwindling steadily.

Only limited conclusions can be drawn from the data on residue analysis (Table 2), but it is apparent that all three insecticides survived the peak-heating process. BHC decreased little during the period of 19 weeks and although the residues for DDT were unaccountably much higher than the original doses DDT must be presumed to have been completely persistent. The concentration of diazinon, however, decreased by over half during peak-heating and fell almost to zero between the fifth and twelfth weeks.

BHC and aldrin completely controlled cecid larvae (Fig. 1) and only with the lower concentration of BHC (100 p.p.m.) did numbers rise to a statistically significant level. Diazinon was also effective initially but larval populations increased steadily throughout cropping. DDT at 100 p.p.m. had virtually no effect

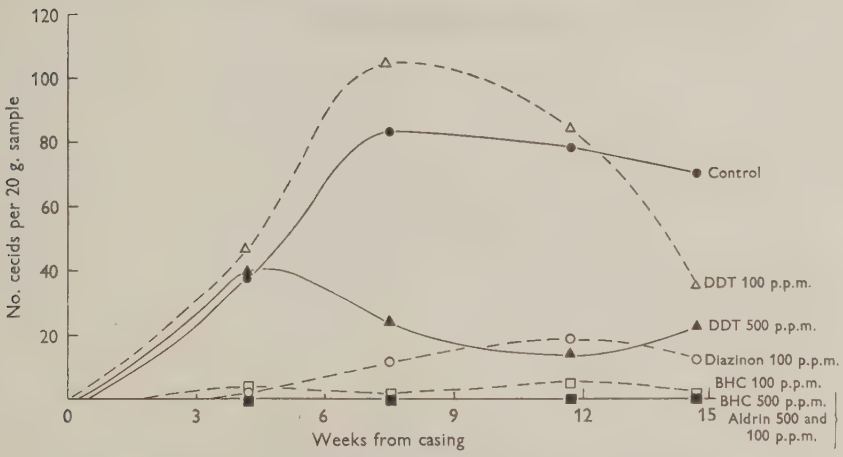

Fig. 1. Effect on *Heteropeza* populations of insecticide dusts mixed with compost.

whatever and the decrease in larval numbers after $7\frac{1}{2}$ weeks in plots treated at 500 p.p.m. was possibly due to a deleterious effect of the insecticide on the mycelium, so causing a gradual diminution of available larval food.

Incorporation of insecticides as emulsions in compost

It was concluded from Exp. 1, (*a*) that the concentrations of insecticides should be reduced, and (*b*) that it would be more convenient to mix the chemicals with the compost as sprays. BHC is cheap and relatively efficient so it was decided to determine the effect of concentrations of this material below 100 p.p.m. (Exp. 2). Another experiment was done to extend the search for an insecticide less toxic to mushroom mycelium by using other chemicals at a concentration of 10 p.p.m. (Exp. 3).

Experiment 2

BHC was sprayed on and mixed thoroughly with the compost during the filling of the beds (46 × 27 in.) each of which contained 100 lb. of compost. The emulsions at concentrations of 1, 2, 5, 10, 20, 50 and 100 p.p.m. in 100 c.c. of water were applied with a power-sprayer. Immediately before casing each bed was inoculated with forty larvae each of the two species *Heteropeza pygmaea* and *Mycophila speyeri*. As before, the experiment was laid out to a randomized block design with four replications of each treatment.

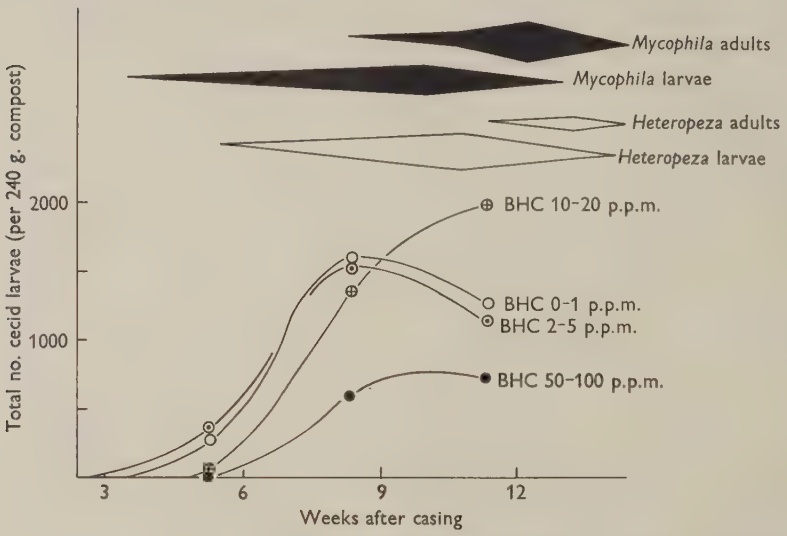

Fig. 2. Effect on cecid populations of different concentrations of BHC. Compare rate of population increase by *Mycophila* and *Heteropeza* and periods of adult flight.

To clarify the presentation of the results, treatment totals for the larval populations have been grouped for pairs of concentrations (0 and 1, 2 and 5 p.p.m., etc.) and the appropriate means plotted (Fig. 2). The initial delay in population growth obtained by incorporating γ-BHC in strengths above 10 p.p.m. occurs during the period of maximum crop production. It will be noticed that after 11 weeks from casing the population in the compost treated with 10–20 p.p.m. BHC was double that in the composts treated with lower concentrations. This was probably due to the starvation effects initiating migration in those plots in which population increase had commenced soon after infection. Fig. 2 also illustrates the relation between the development of larval populations by paedogenesis and the appearance of adult flies in the light-trap. *Mycophila* developed more rapidly than *Heteropeza*, and in fact reached a peak 5 weeks earlier, so that emigration from beds occurred at an earlier stage of cropping. As the larvae of *Mycophila* are orange they are more

obvious than those of *Heteropeza* on mushrooms, and so are economically more important.

There were no significant differences between the yields of the control plots and those of any treatment.

Experiment 3

The same method of application was used to incorporate emulsions of the following insecticides in the compost at a concentration of 10 p.p.m.: γ-BHC, rogor, malathion, dieldrin, aldrin, dipterex and dimetan solution. The same experimental design was used but as the plots contained only 50 lb. of compost the beds were inoculated with only twenty larvae of each species.

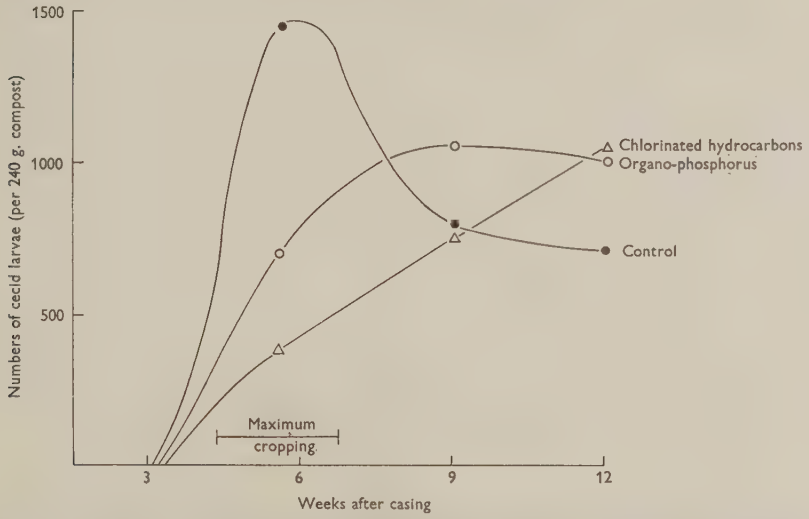

Fig. 3. Control of cecid populations by insecticides. The cecid populations are based on the combined totals for both species and the insecticide groups are each based on three compounds (see text).

Again there was no statistical significance in yield differences between treated and control plots (Table 3). The larval sampling revealed differences in the rate of population increase similar to those found in Exp. 2. The degree of control exerted by the insecticides is most conveniently illustrated by combining the populations for the two cecid species and grouping the chemicals as organo-phosphorus or chlorinated-hydrocarbon compounds (Fig. 3). This graph is based on the geometric mean of larval populations on the sampling dates indicated. The organo-phosphorus group includes rogor, malathion and dipterex, while the chlorinated-hydrocarbon group includes BHC, dieldrin and aldrin. Dimetan, a carbamate, was the least effective of any of the compounds and is not included. A striking difference between the increase of larval population in aldrin- and dieldrin-treated plots was observed.

TABLE 3. *Total yields, numbers of mushrooms and cecid populations of beds treated with insecticidal emulsions at 10 p.p.m.* (Exp. 3)

Insecticide	Control	BHC	Rogor	Malathion	Dieldrin	Aldrin	Dipterex	Dimetan	Sign. Diff. 5 %
Total yield (g.)	13,998	11,178	14,095	14,661	12,862	11,106	14,052	14,688	4,588
Total no. mushrooms	1,197	901	1,290	1,326	1,078	1,108	1,332	1,174	—
Mean wt./mushroom (g.)	11·7	12·4	10·9	11·1	11·9	10·0	10·6	12·5	—
No. larvae/30 g. (*n*) 7 weeks after spawning	1,460	264	447	620	371	405	788	869	—
Σ log (*n*+1)	10·07	6·47	8·05	8·22	6·89	6·02	8·97	9·31	2·50

TABLE 4. *Residues of* BHC (*mg. per sq.ft.*) *applied to bed surface before and after casing*

	Before casing								After casing											
	Dust				Emulsion				Control				Dust				Emulsion			
Depth zone (see text) ...	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*
5 days after casing	57	—	2·1	—	59	—	0·5	—	1	1	—	—	39	13	4	5	54	5	1	3
21 days after casing	93	—	0·6	1·0	111	—	0·8	0·9	1	0·9	0·3	0·6	101	6	0·7	0·9	54	3	0·7	1
56 days after casing	115	—	0·9	2·6	76	—	1·1	1·1	4	1	1	0·9	82	9	1	1	85	4	1	1

The two compounds exerted a similar degree of control on *Heteropeza* for about the first 7 weeks after casing, but subsequently the populations in plots treated with aldrin rose steadily to the highest level recorded. No such difference occurred in the control of *Mycophila*. Insecticides seemed to be generally more effective against *Mycophila* than *Heteropeza*.

The chlorinated hydrocarbon compounds were most effective in delaying population increase (Fig. 3). In treated composts the peaks of population were delayed until late in the cropping period and the maximum populations were lower than in untreated compost. This was probably due to the lower nutrient status of senescing mycelium.

Beds treated with malathion and dimetan produced heavier crops than the untreated controls (Table 3), a difference which was even more pronounced if yields were analyzed on a weekly basis, during the maximum cropping period, when the dipterex- and rogor-treated plots also yielded more than the controls. Cecid populations in the beds treated with these compounds were, however, almost as great as those in the controls. Unpublished data from other experiments showed that very large populations (1000 larvae per 20 g. sample $\equiv \frac{1}{4}$ million/sq. ft.) failed to reduce the yield significantly. It is possible, therefore, that the compounds mentioned above actually stimulated mushroom production, although the weight of the individual mushroom was apparently unaffected.

Application of γ-BHC *as a surface layer to the compost or casing*

Experiment 4

The object of this experiment was to determine (i) the persistence of a layer of γ-BHC applied (*a*) before and (*b*) after casing, as a dust or emulsion, and (ii) the degree of subsequent downward penetration as a result of watering and other cultural operations. A randomized block layout was employed with three replications of the treatments on plots 46 × 27 in. The dust, a 1% commercial γ-BHC, was applied at a rate of 104 mg. active ingredient per sq.ft. ($\equiv$ 4 oz./1000 sq.ft.). The emulsion, made up with 0·9 g. γ-BHC in 5 c.c. of acetone, 5 c.c. redistilled 100/120 petroleum ether and 0·9 g. of anhydrous cyclohexylamine dodecyl sulphate added to 500 c.c. water, was applied with a hand pressure sprayer at the same concentration (104 mg./sq.ft.). The controls were sprayed with a similar formulation, omitting BHC.

Samples taken from the compost with corers were cut into sections as follows: (*a*) casing, (*b*) superficial compost layer, (*c*) middle compost layer, and (*d*) bottom compost layer. These were extracted with chloroform and evaporated gently after adding glacial acetic acid. The residues were analysed by the Schecter–Hornstein process using a simplified dechlorination procedure as suggested by Hancock & Laws (1955).

Although BHC residues were sometimes low (Table 4) the results showed that no deep penetration of BHC occurred and that the insecticide remained as a layer at, or near, the surface throughout cropping.

Statistical analysis of the yield data (Table 5) showed that there were no significant differences between the treatments but, where γ-BHC was applied as an emulsion after casing, significantly ($P = 0{\cdot}05$) larger mushrooms were produced than in any other treatment except application as dust after casing. A similar effect was reported by Moreton (1955) when pyrethrum/piperonyl butoxide dust was applied to bed surfaces at $2\frac{3}{4}$ lb./1000 sq.ft.

Table 5. *Total yields from beds treated with* BHC *before or after casing*

Treatment	Total yield (g.)	No. mushrooms	Mean wt./ mushroom (g.)
Dust before casing	25,066	1746	14·4
Emulsion before casing	28,339	1870	15·2
Dust after casing	19,758	1243	15·7
Emulsion after casing	23,393	1172	20·9
Solvent and emulsifier only	18,265	1387	12·5
Sign. diff. 5%	—	702	5·45

Insecticide residues in mushrooms grown on treated compost

Moreton (1955) reported that mushrooms grown on compost treated with malathion at 89 p.p.m. showed no measurable trace of insecticide. During the present work Mr E. D. Chilwell of Fisons Pest Control Ltd., kindly examined mushrooms grown on beds treated with rogor 40 at 10 p.p.m. and could detect no measurable residue. We examined mushrooms from beds in which 500 p.p.m. of DDT had been incorporated in the compost and found a residue of 0·6 p.p.m. This result supports a statement by Mr F. C. Atkins (*in litt.*) which refers to Weigel finding 0·3 p.p.m. of DDT in mushrooms from beds on which 50% DDT has been applied at a rate of 20·8 g./100 sq.ft. after casing. No residue tests were made on mushrooms grown in compost treated with BHC, aldrin or dieldrin, but no mushrooms from these treatments had any taste taint.

Discussion

Incorporation of insecticides into mushroom compost at rates above 100 p.p.m. caused considerable yield reductions whereas at 10 p.p.m. no significant decreases occurred, although yields were so variable that differences less than 20% were not significant. The differences between concentrations may have been influenced partly by the compost and general health of the crop: in Exp. 1, which was grown on a poor synthetic compost, yield was very low (less than 1 lb./sq.ft.), whereas Exp. 3, on a good quality horse-manure compost, yielded an average of 1·8 lb./sq.ft. It is possible, therefore, that a good quality compost may confer on the mycelium a greater resistance to phytocidal effect.

BHC, malathion, and diazinon may safely be mixed with normal composts before the peak-heating process, at concentrations of about 10 p.p.m. without endangering yield. Furthermore, γ-BHC and DDT can be incorporated in this way and probably persist unchanged throughout the cropping period. Diazinon was affected by peak-heating and disappeared 5 weeks after filling; Wood (1956)

recommended the use of this compound at about 67 p.p.m. and stated that it would remain effective after 48 hr. peak heating.

Our data on DDT and rogor confirm that absorption of insecticide from the compost into the mushroom itself is minimal and there is, therefore, unlikely to be a health hazard preventing the use of BHC, malathion or diazinon within composts at rates below 50 p.p.m.

When these experiments were designed it was assumed that the most important economic effect of cecids would be yield reduction, but when the results were analysed no such reductions could be correlated with cecid numbers even when the larvae numbered $\frac{1}{4}$ million/sq.ft. These observations make it difficult to understand how cecids could have been primarily responsible for the disasters attributed to them by the industry a few years ago.

Growers are becoming concerned about the losses of marketable mushrooms arising from migration of the larvae on to the caps and/or gills. We recorded the date and plot on which larvae appeared on the mushrooms, but not numbers of mushrooms so affected. However, there was obviously a tendency for greater loss with larger cecid populations. Large-scale larval migration took place after mushroom production had passed its peak but before the adult cecid flies emerged (Fig. 2), and was possibly, therefore, influenced (*a*) by the phase of mycelial development, and (*b*) by cecid population density. These effects may be very localized as damage also occurred in beds with small cecid populations. It is relevant here to stress that within the compost habitat cecid larvae do not appear to have any predators or competitors to complicate the interaction of pest and mycelium.

Whereas contamination of sporophores by the more conspicuous and rapidly multiplying *Mycophila* larvae make this the more important pest, *Heteropeza* remains a potential danger because it feeds on the gills and may disseminate bacteria.

Our experiments show that within the range of materials tested γ-BHC achieved the best control of both cecid species and that a concentration of about 50 p.p.m. γ-BHC will decrease and delay population increase without depressing yield. As larval migration onto the mushrooms can occur when cecids are few, an insecticidal barrier in the casing layer may provide practical control. Such a barrier did not affect yield and the layer remained intact throughout the cropping period. Information on the efficiency of such an insecticidal layer as a barrier to the upward migration of cecid larvae will be published in a subsequent paper.

We are grateful to Miss B. Gurney for technical assistance and to Dr L. Broadbent for advice in the preparation of the manuscript.

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INSECTICIDAL CONTROL OF PAEDOGENETIC CECID LARVAE IN MUSHROOM BEDS

II. INCORPORATION OF INSECTICIDES IN THE CASING LAYER

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(With 1 Text-fig.)

Aldrin and γ-BHC, at concentrations above 10 p.p.m., were shown to be promising materials in experiments on the prevention of sporophore contamination by cecid larvae, following the incorporation of insecticides in the casing before its application to the beds.

Introduction

Hussey, Wyatt & Hughes (1960) showed that there was no statistically significant reduction in crop even when large populations of cecid larvae developed in mushroom compost. However, the larvae may wander from the compost and contaminate the sporophores. The orange larvae of *Mycophila speyeri* Barnes and *M. barnesi* Edwards are conspicuous and difficult to wash or brush off so they make the mushrooms unmarketable. *Heteropeza pygmaea* Winn. larvae which are white, and therefore less conspicuous, often invade the sporophores and induce bacterial decomposition of the gills and cap surface.

As contamination of the sporophores can occur at low population densities, limitation of the larval population by incorporating insecticides in the compost is not a practical control method, because the use of sufficient concentrations of insecticides to eliminate the larvae inevitably depresses yield. Hussey *et al.* also demonstrated that when γ-BHC was applied to the surface of the casing or compost, either as a dust or emulsion, no deep penetration of the insecticide took place and the layer persisted at, or near, the surface throughout cropping. Growers have often attempted to prevent larval migration by applying BHC dusts to the bed surface, but with poor results probably because the larvae still had access to the bases of the stipes below the casing surface. It seemed possible that if insecticide could be incorporated throughout the casing layer it would provide a barrier through which the cecid larvae would not pass.

Methods

For Exp. 1, trays (3 × 2 ft.) were filled with horse manure compost prepared under normal commercial practice and laid out in the cropping house to a double Latin square design giving eight replications of the following four treatments which were applied to the casing (a 50/50 (v/v) peat/chalk mixture): (*a*) untreated control; (*b*) γ-BHC incorporated as an emulsion at 100 p.p.m. using a spray-gun;

(*c*) malathion similarly incorporated at 100 p.p.m., and (*d*) a 5% emulsifiable pyrethrins synergized with 50% piperonyl butoxide w/v sprayed on the casing surface at weekly intervals at a rate of 1·2 g. per plot.

Ten *M. speyeri* larvae were introduced into the centre of each plot 2 days after spawning with grain spawn.

At intervals cecid larvae were extracted from samples of compost by the Baermann funnel technique (Hussey *et al.* 1960) to determine the population in the compost. As the mushrooms were picked they were examined individually and if a single larva were visible they were classified as contaminated.

For Exp. 2, the trays were laid out in an 8 × 8 ft. Latin square and innoculated the day after spawning by sprinkling water containing eight *M. speyeri* larvae over the bed surface. The casing was treated as follows: control, γ-BHC at 100, 50, 20 and 10 p.p.m. and aldrin at 50, 20 and 10 p.p.m. The crop records again distinguished between contaminated and clean mushrooms and periodic estimates of the larval population were made.

Results

Experiment 1

The extractions showed that the cecid larvae were numerous in the control plots, but significantly fewer in the plots with BHC-treated casings (Table 1). The mushrooms on the plots cased without treatment were severely contaminated

Table 1. *Larval populations in beds with different insecticidal treatment of casing* (Expt. 1)

Numbers of cecid larvae per 10 g. compost at different sampling dates

Days after casing ...	17	42	62	Overall totals
Control	69	397	271	737
γ-BHC 100 p.p.m.	42	55	31	128
Malathion 100 p.p.m.	121	117	161	399
Synergized pyrethrins (spray)	157	193	166	516

Table 2. *Effect of casing treatment on total yield, proportion of crop contaminated, and weight of individual mushrooms* (Exp. 1)

Treatment	Total yield (g.)	Contaminated yield (g.)	Yield (%) contaminated	Wt./mushroom (g.)
Control	32,923	21,891	66·2	9·1
γ-BHC 100 p.p.m.	31,925	337	1·1	10·3
Malathion 100 p.p.m.	32,814	5,756	17·5	9·5
Synergized pyrethrins (spray)	28,844	5,134	17·8	10·1

throughout the cropping period (Fig. 1), the overall loss being 66%. The treatments produced different degrees of control, BHC giving almost complete protection (Table 2). Weekly applications of synergized pyrethrins produced an unexpected cumulative effect by which the loss was reduced from over 50% to less than 5% between the first and fourth weeks of cropping. On the other hand the efficiency of malathion appeared to decrease as cropping progressed. As the overall losses on

both malathion- and pyrethrin-treated plots were 18% they cannot be considered for practical control. There was no significant difference between the total yields of the treated and control plots.

Growers have commented that insecticides tend to delay cropping and so an attempt was made to calculate the date of the periodic flushes from the daily yield data by means of sliding averages. This revealed an average delay of 3 days where insecticide was mixed with the casing before use (Table 3).

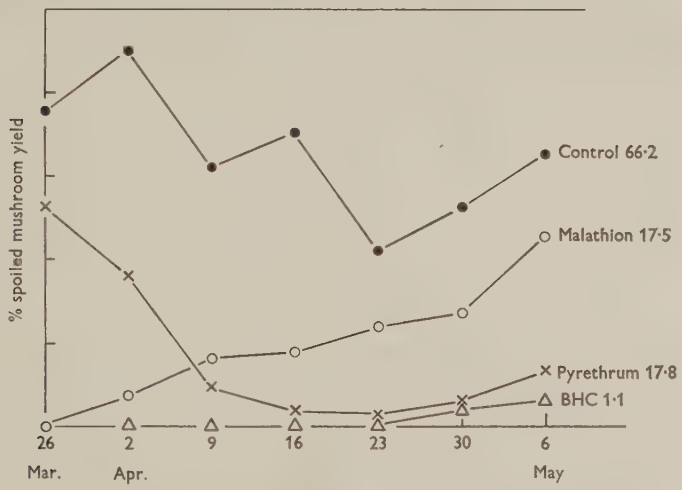

Fig. 1. Proportion of contaminated yield on different treatments over the whole cropping period.

TABLE 3. *Mean delay in flushing dates on plots with different insecticidal treatment of the casing* (Exp. 1)

Treatment	Mean delay in first four flushes (days)
Control	0
γ-BHC	3·2
Malathion	3·0
Synergized pyrethrin	0·2

Experiment 2

Larval sampling indicated unexpectedly low populations compared with Exp. 1, but the percentage of contaminated mushrooms was still considerable on the control plots (Table 4). On all treated plots, however, almost complete control was achieved. Although there was no statistical significance between the yields of the control plots and those with treated casings the yields from beds cased with higher concentrations of insecticides were smaller than those with lower concentrations. However, numbers of mushrooms were significantly fewer than the controls on the

beds cased with higher concentrations of BHC. This is reflected in an increased weight per mushroom, a similar result to that reported by Moreton (1955) and Hussey *et al.* (1960).

Table 4. *Effect of casing treatment on total yield, proportion of crop contaminated, and mean weight of individual mushrooms* (Exp. 2)

Treatment	Total yield (g.)	Total no. mushrooms	Wt./mushroom (g.)	Contaminated yield (g.)	Yield contaminated (%)
Control	40,063	4,609	8·7	18,955	47·3
γ-BHC (p.p.m.)					
100	36,880	2,735	13·5	0	0
50	42,957	3,417	12·5	50	0·1
20	42,835	4,167	10·4	845	2·0
10	43,305	3,907	11·5	105	0·2
Aldrin (p.p.m.)					
50	42,060	4,183	10·2	0	0
20	44,237	4,171	10·6	10	0
10	47,008	4,425	10·5	45	0·1

Discussion

Larvae usually contaminate sporophores as a result of random wandering and although the number infesting the mushrooms is proportional to the population in the compost, in practice, commercial loss results even if only a few larvae are present on a single mushroom. This wandering appears to be stimulated by routine watering of the beds and may occur even when few larvae are present. As the population increases the number of contaminated sporophores also increases until the crop may become a total loss.

The rate of population increase differs in the three cecid species: in *Mycophila speyeri* the larval population may become maximal in the first few weeks of cropping and then decline. Populations of *M. barnesi* develop more slowly and so cause losses later in the crop. *Heteropeza* takes still longer to reach its peak population and so affects the mushrooms at the end of the cropping period. By introducing different numbers of *Heteropeza* larvae into experimental beds at different stages of mycelial development it was shown that the populations rose logarithmically at a constant rate until about 9 weeks after spawning when the rate of increase fell abruptly. This suggested an alteration in the nutrient value of the mycelium at about the end of the peak period of cropping presumably due to a change in the physiological activity of the mycelium. At this time watering may, in this species, induce a mass migration from the compost on to the bed surface. These migratory larvae tend to move towards light and if they encounter a dry surface they aggregate in writhing masses often as large as a sixpence. A mushroom in the path of such a migration will become completely enveloped with larvae.

Preliminary studies have been made on the tropisms initiating larval movement. It has been shown that cecid larvae, in a water film equal to about half the body thickness, are positively phototropic and negatively geotropic. On dry surfaces

less directional wandering takes place and there is a tendency for the tropisms to be reversed. It appears therefore that larvae can only wander freely towards the bed surface when adequate water films are present such as after watering. The mass migration referred to in *Heteropeza* is probably a similar response initiated by a change in the nutrient status of the mycelium.

Incorporation of γ-BHC in the casing, immediately before its application to the beds, at rates of 10 p.p.m. drastically reduced sporophore contamination on heavily infested beds. As spent mushroom compost is often used on horticultural land, where BHC may cause taint in sensitive root crops, aldrin seems the most promising material. Practical experience suggests that the insecticide is most easily mixed with the damp peat by applying, as an emulsion, in as small a quantity of water as possible consistent with achieving an even distribution.

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STUDIES ON THE PHOTOLYSIS OF PYRETHRUM

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The rates of photolysis of films of pyrethrum were studied to determine the factors which could contribute to their increased stability.

The claims that the green colouring matter (referred to as chlorophyll) present in crude oleoresin contributed to the rapid photolysis of pyrethrum films were verified. Evidence was accumulated to show that light of a wide range of wavelengths could effect photolysis.

The action of *para*-aminoazobenzene as a stabilizer was studied in detail and the experimental evidence obtained strongly suggested that this compound did not exert its action by virtue of its light-absorbing properties but as a direct or indirect inhibitor of a chemical reaction. Assays of the four active principles in extract before and after irradiation showed that the PAAB protected primarily the cinerin II and pyrethrin II.

Sudan IV and *p*-methoxyazobenzene and Spirit Red were shown to have similar stabilizing properties to PAAB.

The current increase in demand for insecticides which do not leave toxic residues on plant or animal foodstuffs for human consumption has resulted in renewed attention to the possibility of retarding the rapid photolysis of pyrethrum in sunlight.

This work was undertaken following the publication of U.S. Patent 2,772,198 (1956) claiming the stabilization of pyrethrum films by *para*-aminoazobenzene.

The experiments described in this paper were made to assess the relative importance of the various factors concerned with the degradation of pyrethrum films in sunlight and the action of certain chemical stabilizers described in the literature. Furthermore, it was hoped that the data presented would be of use to other workers wishing to screen potentially useful stabilizers.

For reasons elaborated later, the various data listed in this paper should be considered qualitatively rather than quantitatively.

Tattersfield & Martin (1934) found that ground pyrethrum flower heads and pyrethrum-impregnated talcs lost their biological activity when irradiated with either sunlight or tungsten light. The photolysis occurred more rapidly in air than in nitrogen. The addition of antioxidants, hydroquinone and tannic acid slowed down the rate of photolysis.

Nasir (1953) summarizing his own work and that of Blackith (1952*a*, *b*, *c*) suggested that three distinct chemical inactivation reactions were involved when films of pyrethrins were exposed to ultra-violet light.

(1) A rapid direct photolysis, independent of the presence of oxygen, when the u.v. light in the range 2100–2500 Å is absorbed by the insecticide molecules.

(2) A slower and more general light-catalyzed decomposition.

(3) A much slower oxidation reaction which is independent of the presence of light.

Nasir (1953) showed that a significant loss of biological activity occurred when films were exposed to light of wave-lengths ranging from 2500–4000 Å which are not absorbed by pyrethrin molecules, though he considered the small proportion of u.v. below 2500 Å in solar radiation to be largely responsible for the rapid photolysis of pyrethrins.

Blackith (1952*b*) showed that benzene-azo-β-naphthol (hereafter referred to as BABN), which absorbs strongly from 2000 to 2800 Å, partially protected pyrethrins from photolysis though the degree of protection so afforded has not been considered sufficient to warrant the inclusion of the dyestuff in commercial formulations.

Blackith (1952*c*) showed that pyrethrins were less stable than allethrin when exposed to u.v. light and BABN had less effect as a protectant with films of the synthetic homologue of cinerin I than with the natural esters.

Brown & Phipers (1955) observed that when films prepared from a commercial sample of pyrethrum oleoresin extract were irradiated with tungsten light, changes occurred in both the acidic and alcoholic portions of the molecule. Certain plant pigments collectively referred to as 'chlorophyll' appeared to have a catalytic effect on the degradation of chrysanthemic acid. The irradiation also induced slower changes in the cyclopentenolone system and this reaction was found to be independent of the presence of 'chlorophyll'. The addition of hydroquinone retarded both types of reactions which suggested that they were oxidative in character.

Freeman (1956) using a purified pyrethrins concentrate also demonstrated that under u.v. light the natural esters degraded more rapidly than allethrin. It appeared that initially the pyrethrins only were affected leaving the cinerins unchanged. His interpretation of a detailed examination of the infra-red spectrum of degraded purified pyrethrins was that the only change occurring in the pyrethrin molecule occurs at the *cyclo*pentenone side chain.

Brown, Hollinshead, Phipers & Wood (1957) using a method of assay which separated the four esters quantitatively showed that under irradiation from tungsten lamps.

(1) The pyrethrin I and cinerin I in crude oleoresin degrade before the pyrethrin II and cinerin II.

(2) The cinerins are more stable than the pyrethrins.

(3) The removal of the green colouring matter resulted in a greater stability of the pyrethrin I and cinerin I, therefore proving that the presence of these pigments accelerated the decomposition of chrysanthemic acid.

(4) Oleoresin freed from chlorophyll was given a further measure of protection from photolysis when hydroquinone was added.

Experimental

Preparation of films

Except where stated otherwise, the films were prepared by pipetting 0·5 ml. of the test solution normally containing 0·25 mg. of pyrethrins dissolved in ethanol on to the centre of a 10 × 10 cm. glass plate and allowing the pool of liquid to spread by

rocking the plate. As the solvent evaporated, the film was gently brushed to make it as even as possible.

Irradiation conditions

(1) *Sunlight.* The experiments were conducted at Nakuru 30 miles south of the Equator and 6100 ft. above sea level. The plates were irradiated in direct sunlight with clear skies. It can be safely assumed that under these conditions, with no smoke haze, the proportion of the total radiation in the u.v. fraction was considerably in excess of that normal at sea level in subtropical latitudes. The intensity of radiation was probably of the order of 90 cal./cm.^{2}.

(2) *Tungsten light.* Two 150 W. opal bulbs set in metal reflectors with the centre of the filament 2 ft. 6 in. from the surface irradiated.

Biological assays

Films. In order to permit experimentation involving a large number of replicates and permutations, a simple method of bioassay involving the primary response to pyrethrum (commonly referred to as 'knock down') only was used. Ten adult *Tribolium castaneum* Hbst. were confined on each glass plate with a 9 cm. glass ring which the beetles were unable to climb. After a 10 min. exposure period, those beetles lying on their backs and unable to right themselves were recorded as paralysed (hereafter referred to as KD). In certain experiments, the time taken for the KD of all the beetles was used as the assay response.

Dusts. Dusts were assayed using the wheat/grain weevil *Calandra oryzae* L. technique or Parkin (1951) modified by Goodwin-Bailey & Holborn (1952).

Sources of toxicants

Crude oleoresin: commercial sample containing 23·6% pyrethrins by method of Association of Official Agricultural Chemists (1955). A free-flowing opaque liquid containing a range of pigments normally associated with chlorophyll.

Refined oleoresin: commercial sample containing 20% pyrethrins *A.O.A.C.*, 8th edition. Pale-orange transparent liquid. Refined by filtration through decolorising charcoal.

Dried flowers: clonal material ex-Kenya.

Allethrin: laboratory-refined sample containing 93·4% synthetic homologue of cinerin I.

(1) *Comparison of rates of degradation of pyrethrum films in sunlight and in darkness*

Six glass plates were coated with 0·25 mg. of pyrethrins. Three were exposed to sunlight for successive periods, of 10, 20 and 40 min., and biologically assayed between each exposure. The remaining plates were stored at 28° C. in the dark and assayed after 1 week, 1 month and 6 months, respectively. The average percentage of beetles completely paralysed after 10 min. exposure is shown in Table 1 and the data indicates that the loss of biological activity in sunlight was extremely rapid whilst films stored in the dark were stable for at least 4 weeks.

(2) *The effect of excluding the ultra-violet radiation from sunlight on the rate of photolysis of pyrethrins*

Two filters were used: a 3 mm. sheet of 'green' window glass that absorbed radiation of wave-lengths less than 3400 Å and a 0·05 in. sheet of polymethylmethacrylate impregnated with a commercial u.v. absorber Uvinul D-49 which absorbed radiation of wave-lengths less than 3960 Å.

TABLE 1. *Rates of loss of biological activity in sunlight and darkness*

Sunlight		Darkness	
Exposure time (min.)	Percentage KD in 10 min.	Exposure time (weeks)	Percentage KD in 10 min.
10	85	1	100
20	45	4	100
40	10	26	55

TABLE 2. *The effect on pyrethrum photolysis of interspersing two u.v. filters in sunlight*

		Percentage KD in 10 min.		
Filter	Irradiation time (min.)	Filtered	Non-filtered	Control not irradiated
Window glass	20	85	53	100
	40	45	28	100
	60	24	8	100
Plastic sheet impregnated with Uvinul 49-D	10	95	65	100
	30	75	55	100
	50	52	6	100

The filters were arranged so that only sunlight passing through them could reach the films of pyrethrins prepared as described on p. 353. Three replicates were used under each filter and a further three plates exposed to full sunlight at the same time. The assays after each exposure interval are shown in Table 2 and indicate that whilst the u.v. portion of the sun's radiation does have a significant effect on photolysis the visible radiation is also capable of effecting degradation.

The results must, however, be interpreted with caution as the data obtained with the screens in the spectrophotometer might not be wholly applicable in this high intensity of sunlight.

(3) *The effect on the rate of photolysis of the partially decolorized pyrethrum oleoresin*

Replicated films were prepared as previously to compare:

(*a*) Crude oleoresin.

(*b*) Commercial refined orange-coloured extract.

(*c*) Extract type (*a*) refined by filtration through Fuller's earth to give a pale orange solution.

The three groups of films were irradiated in sunlight simultaneously and the rates

of degradation as revealed by bioassays are shown in Table 3. The removal of the colouring matter normally associated with chlorophyll resulted in a marked increase in stability.

(4) *Comparison of the rates of photolysis of allethrin and pyrethrin*

Using the technique noted on p. 353, films of pyrethrins prepared from refined (with charcoal) and non-refined extract were compared with allethrin. The results, Table 4, suggest that allethrin was more stable in sunlight than non-refined extract and that it was of the same order of stability as the decolorized pyrethrum extract.

TABLE 3. *The rates of photolysis of films of refined and non-refined pyrethrum extract in sunlight*

	KD 10 min.		
Irradiation time (min.)	Crude oleoresin extract (%)	Commercial refined orange-coloured extract (%)	Extract (*a*) refined (%)
30	35	100	100
60	8	85	72

TABLE 4. *Comparison of the rates of photolysis of allethrin with refined and non-refined extract*

	Percentage KD in 10 min.		
Irradiation time (min.)	Allethrin	Refined extract	Non-refined extract
0	100	100	100
30	100	100	63
60	58	50	12

TABLE 5. *The effect of* PAAB *on the rates of photolysis of refined and non-refined pyrethrum extract*

	Percentage KD in 10 min.			
Irradiation time (min.)	Refined extract (*a*)	Refined extract + PAAB (*b*)	Non-refined oleoresin (*c*)	Non-refined oleoresin + PAAB (*d*)
30	100	100	64	100
60	58	100	11	85
90	25	100	15	52
120	5	100	12	10
150	12	95	5	8

(5) *The protective action of* para-*aminoazobenzene* (*hereafter called PAAB*)

Replicates of the following films were irradiated simultaneously: (*a*) Charcoal refined extract. (*b*) Refined extract and PAAB in a 1 : 1 ratio, i.e. 0·25 mg. pyrethrum and 0·25 mg. PAAB. (*c*) Non-refined oleoresin. (*d*) Non-refined oleoresin plus PAAB.

The biological data given in Table 5 demonstrate clearly the increased stability afforded by the addition of PAAB and show that this would appear to be additive to the effect produced by the removal of the chlorophyll.

(6) *The effect of* PAAB *on the rate of photolysis of allethrin*

Allethrin exerts a somewhat slower KD response than pyrethrum and when PAAB is added at a 1:1 ratio the rate of paralysis is further retarded, probably because of a physical effect on the film surface. To obtain a valid comparison of the rates of photolysis, the time interval before KD was recorded was varied. The data in Table 6 show that PAAB did retard the rate of photolysis of allethrin.

TABLE 6. *The effect of PAAB on the rate of photolysis of allethrin*

Irradiation time (min.)	Exposure time of beetles on plates (min.)	KD percentage; allethrin	KD percentage; allethrin + PAAB
0	10	100	83
40	10	98	85
70	20	56	100
100	25	39	78
130	30	42	90

N.B. The fluctuation in the KD results with PAAB might have been due to the crystals of the dyestuff forming on the surface which were disturbed by the successive groups of beetles.

(7) *The effect of adding PAAB to pyrethrum-impregnated dusts*

PAAB was added to a 0·5% (w/w) pyrethrum dust at a 1:1 ratio by (*a*) solvent impregnation, (*b*) dry mixing in a ball mill.

10 g. of each dust was spread out as a thin layer on a glass dish 260 cm.^{2} in area and irradiated under tungsten light for 9 hr. Control samples of dust were stored in amber glass bottles at 40° C., which was the highest temperature reached by the dust during irradiation. The data obtained from subsequent bio-assays (Table 7) show that both powders containing PAAB suffered appreciably less degradation than the pyrethrins alone. Furthermore, it was apparent that the spectrum emitted by tungsten light within opal bulbs was capable of effecting photolysis.

TABLE 7. *The effect of irradiating pyrethrum-impregnated dusts containing PAAB*

Treatment	Percentage weevils dead in 6 days (Av. three replicates)		
Wt. of dust applied ...	0·05 g.	0·1 g.	0·2 g.
Pyrethrum alone control	78	97	100
Pyrethrum + PAAB control (*a*)	63	98	100
Pyrethrum + PAAB control (*b*)	73	95	100
Pyrethrum alone irradiated	4	8	91
Pyrethrum + PAAB (*a*) irradiated	53	95	100
Pyrethrum + PAAB (*b*) irradiated	51	99	100
Control: talc alone	1	0	4

(8) *The effect of using PAAB as a filter for sunlight*

Films of pyrethrins and of pyrethrins containing PAAB were exposed to sunlight filtered through a sheet of 3 mm. window glass which had been sprayed to a deep orange colour with PAAB. A similar group of films were exposed through plain window glass. The data obtained in subsequent biological assays (Table 8*a*) showed that when PAAB was used as a light filter but was not in contact with the pyrethrins it failed to effect an increase in stability.

The test was repeated using filters made by filling flat glass dishes to a depth of 0·5 cm. with kerosene solutions of (*a*) 0·3 % PAAB, and (*b*) 0·3 % BABN. Although the kerosene solutions reduced the intensity of the radiation appreciably, photolysis occurred with all plates which did not have PAAB incorporated with the pyrethrins (Table 8*b*).

TABLE 8*a*. *The effect of filtering the sun's radiation with PAAB as a film on glass*

	Percentage KD in 10 min.			
	Glass sprayed with PAAB		Plain glass	
Irradiation time (min.)	Pyrethrum alone	Pyrethrum + PAAB	Pyrethrum alone	Pyrethrum + PAAB
40	37	100	43	100

TABLE 8*b*. *The effect of filtering the sun's radiation with kerosene solutions of PAAB and BABN*

PAAB filter		BABN filter	
Pyrethrum alone	Pyrethrum +PAAB	Pyrethrum alone	Pyrethrum +PAAB
65 %	100 %	48 %	100 %

TABLE 9. *The protective effect of PAAB against heat degradation*

	Time (min.) to achieve 100 % KD	
Treatment	Pyrethrum alone	Pyrethrum +PAAB
Films as prepared	4½	5
Heated at 100° C. for 1 hr.	12	17
Irradiated for 5 hr. subsequent to heating	210	42

(9) *The effect of heat and sunlight on films of pyrethrum in the presence and absence of PAAB*

It is well known that pyrethrins and cinerins are degraded by prolonged exposure to a high temperature and the following experiment was undertaken to discover whether PAAB retards the rate of thermal decomposition. Films as prepared in previous experiments were heated for 1 hr. at 100° C., bio-assayed and subse-

quently irradiated in tungsten light for 5 hr. The results shown in Table 9 indicate that PAAB has no protective effect on the processes of degradation initiated by heat although, in the same experiment, the PAAB gave appreciable protection against irradiation.

(10) *The effect on the rate of paralysis of* Tribolium *beetles of adding PAAB to a film of pyrethrum*

Glass plates were coated with 0·5 ml. of each of eight solutions ranging from 0·025 to 0·001% pyrethrins and a parallel series was prepared containing PAAB in addition to the pyrethrins at a 1:1 ratio. The plates were stored in the dark for 24 h. before undertaking the bioassays and the response measured was the time taken for 90% knock-down of the beetles on the plates. Two replicates were used for each concentration.

The results, Table 10, show that the presence of the PAAB slowed down the rate of paralysis: this was thought to be due to either a slower rate of pick-up or a slower penetration of the toxicant.

Parallel results were obtained with other stabilizers but the degree to which the rate of paralysis was retarded varied with individual compounds.

It would, therefore, appear difficult to effect a quantitative appreciation of the data in most of the previous experiments and it is suggested that their interpretation should be only on a qualitative basis.

TABLE 10. *The effect on the rate of paralysis of adding PAAB to a film of pyrethrins*

Concentration ...		0·25%	0·1%	0·05%	0·025%	0·01%	0·005%	0·0025%	0·001%
KD 90% (min.)	Pyrethrum alone	4	5	5·5	6	9	10	10	16
	Pyrethrum +PAAB	6	12	20	35	45	40	60	100

(11) *The protective action of PAAB on the four insecticidal constituents of pyrethrum extract*

20 g. of ground-dried pyrethrum flowers were extracted with petroleum ether (B.P. 40–60° C.) and aliquots containing approximately 20 mg. active ingredients were treated as follows:

(*a*) Stored in the dark in solution.

(*b*) Evaporated in a 9 cm. flat bottomed glass dish to give an even dry film.

(*c*) As (*b*) with 15 mg. PAAB added.

(*d*) As (*b*) with 25 mg. PAAB added.

(*e*) Filtered through column of activated charcoal using excess of a (1:2) mixture of diethyl ether and petrol ether. Other experiments had shown that this treatment permitted at least 98% recovery of active ingredients and removed all colour.

The films were irradiated in sunlight and analysed by the charcoal elution chromatographic method of Stephenson (1960). In Table 11 the results are compared with those from the solution stored in the dark.

It is possible to infer from these limited data that the pyrethrins degrade faster than the cinerins; that the decolorization process results in greater stability of all components except pyrethrin II and that the PAAB has its main protective action on the pyrethrin II and cinerin II.

TABLE 11. *The effect of irradiation in sunlight on the four active constituents of pyrethrum extract*

Treatment ...	Percentage of each constituent remaining (weight basis of original flowers) and percentage decomposition relative to control sample			
	Cinerin I (%)	Pyrethrin I (%)	Cinerin II (%)	Pyrethrin II (%)
Control in dark	0·234 (100)	0·509 (100)	0·379 (100)	0·669 (100)
Pyrethrum alone irradiated 20 min.	0·118 (50)	0·219 (43)	0·289 (76)	0·411 (61)
Pyrethrum alone irradiated 120 min.	Nil	Nil	Nil	Nil
Decolorized pyrethrum irradiated 20 min.	0·229 (98)	0·411 (81)	0·377 (99)	0·210 (31)
Pyrethrum + 15 mg. PAAB irradiated 120 min.	0·131 (56)	0·325 (64)	0·385 (102)	0·625 (93)
Pyrethrum + 25 mg. PAAB irradiated 120 min.	0·156 (65)	0·350 (69)	0·376 (99)	0·630 (94)

(12) *A comparison of the stabilizing properties of PAAB, BABN and other dyestuffs*

A direct comparison was made between the rate of degradation of films containing equal concentrations of PAAB and BABN when irradiated in sunlight. The data in Table 12 indicate that the rate of photolysis of pyrethrins was retarded by both dyestuffs, but that PAAB was the more effective compound.

TABLE 12. *The effect of PAAB and BABN as pyrethrum stabilizers*

Irradiation time (min.)	Percentage KD in 10 min.		
	Pyrethrum alone	Pyrethrum + BABN	Pyrethrum + PAAB
15	60	100	100
30	33	30	100
45	15	8	100

A range of dyestuffs and commercial u.v. absorbers were selected at random and compared with PAAB. Their approximate value as stabilizers is listed in Table 13. The absorption characteristics in the wave-length range 2000–6000 Å of each chemical listed in Table 13 was determined using a Unicam S.P. 500 Spectrophotometer. Although PAAB had the most powerful light-absorbing properties it was not found possible to establish a positive correlation between spectral absorption properties and effectiveness as stabilizers of the various compounds tested.

(13) *The effect of the addition of anti-oxidants to films of pyrethrum*

Resorcinol, pyrogallol, catechol and hydroquinone were selected as typical anti-oxidants and added to the pyrethrins (in a 1 : 1 ratio) when the films were prepared.

The data, Table 14, show that all four anti-oxidants improved the stability of the films but not to the extent induced by PAAB. In a further trial no improvement in the degree of stability was obtained when hydroquinone was added to pyrethrum films containing PAAB. Furthermore, hydroquinone was shown to improve the stability of both refined extract and crude oleoresin.

TABLE 13. *Relative effectiveness of a range of dyestuffs and ultra-violet absorbers in retarding the rate of photolysis of pyrethrins*

Assuming PAAB has an effect of 100		Assuming PAAB has an effect of 100	
Sudan IV (*o*-toluene-azo-*o*-toluene-azo-β-naphthol)	94	Kiton Red	35
p-Methoxy-azobenzene	90	Acid Scarlet	28
Spirit Red	86	Fluorescin	20
Hydroxyazobenzene	83	Azobenzene	20
5-Chlor-5-hydroxybenzene-*o*-phenone	40	Benzene-azo-β-naphthol	10
Azo-carmine	40	Sudan Yellow	0
		Uvinul 400	0
		Amaranth	0

TABLE 14. *The effect of anti-oxidants on the stability of pyrethrum films*

	Percentage KD 10 min.					
Irradiation time (min.)	Pyrethrum alone	Pyrethrum + resorcinol	Pyrethrum + pyrogallol	Pyrethrum + catechol	Pyrethrum + hydro-quinone	Pyrethrum + PAAB
10	55	95	100	100	94	100
20	20	70	76	85	89	100
30	55	85	77	56	87	100
40	30	12	25	45	42	100

DISCUSSION

The data from these experiments suggest that the photolysis of pyrethrins can be effected by light of a wide range of wave-lengths and that the use of a dyestuff such as BABN to filter the wave-lengths which are actually absorbed by the pyrethrin and cinerin molecules is likely to have only a very limited effect on retarding the rate of photolysis when pyrethrum formulations are used in daylight.

There appears to be considerable evidence to support a theory that PAAB and the other active compounds listed in Table 14 act as chemical stabilizers possibly as inhibitors of free radicals necessary as intermediates for the photochemical reaction. The fact that the action of the PAAB was additive to the increased stability afforded by the removal of the 'chlorophyll' pigments would appear to be due to the former compounds primarily protecting the pyrethrins II and cinerins II. The data in this paper confirm previous work which showed that the removal of the green colouring matter increases the stability of the pyrethrins I and cinerins I.

PAAB has not to date proved to be suitable for use in commercial formulations owing to its known carcinogenic properties and its tendency to stain foliage, but there would appear to be a considerable possibility of selecting a compound of similar stabilizing properties with a low mammalian toxicity which would be non-staining. Further work on these lines is proceeding.

Although refined pyrethrum extracts have been available in the U.S.A. for several years they have only recently become freely available in other countries and their presence should facilitate the manufacture of pyrethrum formulations which will have a greater degree of stability in sunlight.

I am indebted to Mr H. Stephenson for undertaking the individual assays of the four toxicants, Dr E. A. Baum for determining the absorption characteristics of the various dyestuffs and Miss R. Williamson for valuable assistance with the bio-assays.

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THE EFFECTS OF TEMPERATURE AND HUMIDITY ON THE RATE OF DEVELOPMENT AND THE MORTALITY OF *TRIBOLIUM CONFUSUM* DUVAL (COLEOPTERA, TENEBRIONIDAE)

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(With 7 Text-figures)

The speed of development and the developmental mortality of *Tribolium confusum* were studied over a series of constant temperatures between 15° and 40° C. at 10, 30, 70 and 90 % R.H. using wheatfeed as food.

Eggs did not hatch at 15° or 40° C. at any humidity. At 37·5° C. about 60 % of eggs hatched and at all other conditions about 90 % hatched. The effect of temperature on the duration of the egg period is shown graphically, the shortest period being at 35° C. Humidity does not affect the egg period.

Larvae failed to develop to pupae at 17·5° C., at 10 % R.H. at 20° C., and at 10 and 90 % R.H. at 37·5° C. The rate of larval development was affected by both temperature and humidity being quickest at the higher humidities and at about 32·5° C. Larval mortality was less than 16 % except at 37·5° C., at 10 % R.H. or less, and at 20° C., 90 % R.H.

The duration of the pupal period was not affected by humidity and was shortest at 37·5° C. The total developmental period is compared with that of *T. castaneum* over the range of temperature and humidity conditions in which both species can grow. The optimum for developmental speed and the maximum and the minimum temperatures at which development is possible were all about 2·5° C. lower for *T. confusum* than for *T. castaneum*. The developmental periods for the two species were equal at temperatures between 23 and 27° C., depending on humidity. At lower temperatures, *T. confusum* developed the more quickly and at higher temperatures the more slowly.

Introduction

The confused flour beetle, *Tribolium confusum* Duval, has been used extensively for laboratory studies of ecology and insect nutrition. Consequently a great deal of information is available in the literature on its rate of development, especially on wholemeal flour and similar foodstuffs. There is, however, no study covering the entire range of temperature and humidity conditions at which development is possible, nor has the published data been reviewed comprehensively since Park (1934) wrote his paper on the general biology of the species. This paper is intended to meet these needs and also to compare for *T. confusum* and the closely related *T. castaneum* (Herbst) the rates of development of the various stages in similar conditions when both are grown on wheatfeed.

Experimental methods

The rates of development and mortality were determined at a range of constant temperatures from 15 to 40° C. by steps of 2·5° C. At all temperatures, relative humidities of 10, 30, 70 and 90% were used and a few extra levels were included at particular temperatures. Solid phosphorus pentoxide was used to give '0'% R.H. at 35° C. and distilled water to give '100'% R.H. at 30° C. Saturated solutions of zinc chloride and barium chloride gave relative humidities of approximately 10 and 90%, respectively. All other levels were obtained with solutions of potassium hydroxide of the appropriate specific gravity (Solomon, 1951). The relative humidity in desiccators was checked frequently with an electric hygrometer element.

The standard food used was fine wheatfeed obtained by repeatedly grinding the commercial product to make it pass through a sieve of 60 meshes to the inch. This food was conditioned to the requisite moisture content by exposing it for at least 3 weeks in a thin layer in Petri dishes in desiccators containing solutions giving the desired relative humidity. For 90% R.H. and above, however, the food was conditioned at 70% R.H. but rapidly became mouldy during use, especially at the higher temperatures.

Eggs were obtained at the experimental temperatures, except at 20° C. and at temperatures above 32·5° C. when 22·5 and 30° C. respectively, were used. About 100 young adult beetles were placed at 70% R.H. on a thin layer of fine wholemeal flour in a crystallizing dish about 3 in. in diameter. After 24 hr. eggs were sifted from the flour with a 60-mesh sieve. The beetles were obtained from the P.I.L. insectary stock bred on wholemeal flour at 25° C. and 70% R.H. Although the original cultures were started about 20 years ago, this stock is not highly inbred as individuals collected in flour mills have frequently been added to it.

The eggs were incubated at the various experimental conditions in groups of ten in 2 × 1 in. glass tubes. On hatching, each larva was placed in a 2 × ½ in. glass tube on about 220 mg. of fine humidity-conditioned wheatfeed measured by volume. At least thirty larvae were set up at each combination of temperature and humidity and were left undisturbed except for daily opening of the desiccator until a day or two before pupae were expected. The size of the larvae was then checked by a quick examination of the tubes. When the larvae were considered to be fully grown, the tubes were examined at the same time each day and with the minimum of disturbance to the contents until the larvae pupated, when the food was discarded. On the day the insect became an adult its weight was determined to the nearest 0·1 mg. Sex was determined from the pupa and checked in the adult. This was easily done using the character mentioned by Hinton (1942), because the young adult beetles will remain on their backs in the Petri dish and the pit and hairs on the front femur of the male are, at this age, much darker than the rest of the femur.

The percentage hatch and duration of the egg stage

A very high percentage hatch of eggs was obtained (Table 1) at all relative humidities at temperatures from 17·5 to 35° C., inclusive. At 37·5° C. about 60% of the eggs hatched at each humidity. A similar percentage hatch was obtained by Oosthuizen (1935) at 37·5–38·5° C. No hatching occurred at 40° C., a temperature at which Lin, Hodson & Richards (1954) obtained 27% hatch. No hatching was observed at 15° C., the temperature estimated by Butler (1949) to be the minimum for egg development in *T. confusum*. Neither Khalifa & Badawy (1955*b*) nor Lin *et al.* (1954) obtained any hatching at 16° C. but Lin *et al.* recorded that 21% of eggs

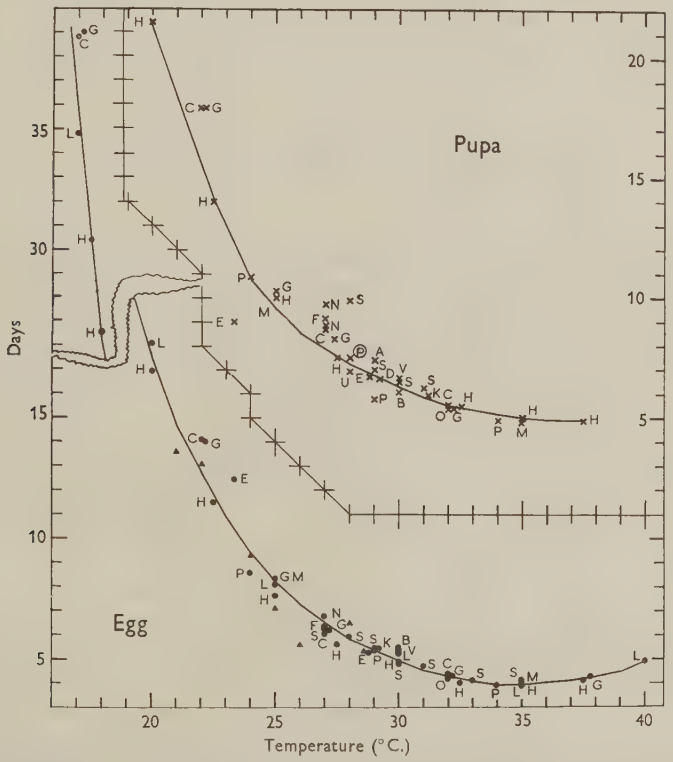

Fig. 1. The effect of temperature on the duration of the egg stage (lower line) and pupal stage (upper line) of *Tribolium confusum* according to various authors. A = Schneider (1943); B = Brindley (1930); C = Chapman & Baird (1934); D = Khalifa & Badawy (1955*a*); E = Estácio (1953); F = Holdaway (1932); G = Gray (1948); H = present work; K = Kennington (1953); L = Lin *et al.* (1954); M = Mickel & Standish (1946); N = Good (1933, 1936); O = Cashman (1952); P = Park & Frank (1948); Ⓟ = Park, Miller & Lutherman (1939); S = Stanley (1939, 1946, 1953); U = Huot, Bernard & Lemonde (1957); V = Miller (1944); ▲ = Khalifa & Badawy (1955*b*).

hatched at 17° C. Chapman & Baird (1934) also recorded hatching at 17° C., and Gray (1948) did so at 63° F. (17·2° C.).

Relative humidity appears to have very little effect on either the percentage of eggs hatching or on the period between oviposition and hatching. Holdaway's results (1932) show a very slight lengthening of the egg period at 27° C. from 6·15 days at 25% R.H. to 6·55 days at 1% R.H.

Table 1. *Percentage hatch and duration in days of the egg period of* Tribolium confusum *at various temperatures*

Temp. (° C.)	Hatch (%)	Egg period (days)
40	0	—
37·5	59	4·1
35	98	3·9
32·5	92	4·0
30	94	4·9
27·5	97	5·6
25	89	7·7
22·5	96	11·5
20	98	16·9
18	87	26·4
17·5	85	30·4

The effect of temperature on the duration of the egg period is shown in Table 1. In Fig. 1 the results of the present work are compared with a selection of the published data, all of which are reasonably consistent as is shown by the curve drawn subjectively through them. Also plotted on the figure and shown as triangles are data taken from Khalifa & Badawy (1955*b*) which also fit well. These authors tabulate the mean egg period recorded each month (from March to November) in uncontrolled conditions and the corresponding mean temperatures have been estimated from their graphs of temperature conditions. Their value for July and August, 4·9 days and 30° C., could not be plotted as it corresponded exactly with that obtained in this work. Oosthuizen (1935) gives 4·7 days for the egg period at 37·5–38·5° C.

The development of larvae on wheatfeed

T. confusum larvae cannot complete their growth in some of the more extreme conditions in which eggs hatch. Thus, at 37·5° C. larvae placed at 10 and 90% R.H. all died, and mortality at 30% R.H. was heavy. No pupae were formed at 17·5° C., nor at 10% R.H. and 20° C., although the threshold for hatching is about 17° C.

The shortest mean duration for the larval stage was recorded at 32·5° C. and 70% R.H. At most temperatures the larval stage was slightly shorter at 90% than at 70% R.H. but at 32·5° C. and above, the growth of mould overwhelmed the larvae at 90% R.H. The duration of the larval stage increased progressively as the relative humidity decreased, but about a quarter of the larvae kept at '0'% R.H. at 35° C. were able to pupate and produce fertile adults. The optimum for rapid growth lies between 30 and 35° C. A summary of the data obtained for the duration of the larval stage and the percentage mortality in that stage is given in Table 2.

There is a considerable bulk of published data on the rate of growth of larvae of *T. confusum*. Much of it concerns inferior foodstuffs and so cannot be compared with the results presented in Table 2. A selection of results from the foodstuffs allowing rapid growth is given in Fig. 2 together with some results from the present work. Most of the data relate to flour or other derivatives of cereals, especially to whole wheat flour which appears, from Fig. 2, to be nutritionally similar to wheatfeed, and when fortified by the addition of dried brewer's yeast a little superior. The shortest larval period recorded is 14 days by Mickel & Standish (1946) at 35° C. and 80% R.H. on whole wheat flour with 5% yeast. Sweetman & Palmer (1928) record 14·6 days for ground husked barley at 31–32° C. and 75% R.H. The unlabelled points for 30° C. and 70% R.H. between V and S in Fig. 2 are taken from Lin & Richards (1952), Brindley (1930), Lipke, Fraenkel & Liener (1954), Miller (1944) and Stanley (1953) for cereal foodstuffs and from Barton-Wright (1941) for a synthetic diet containing yeast.

TABLE 2. *The effect of temperature and humidity on larval mortality and on the duration of larval life in days on wheatfeed*

Temp. (° C.)	R.H. (%)	No. of larvae pupated	Mean larval period	S.E. of mean	% dead	R.H. (%)	No. of larvae pupated	Mean larval period	S.E. of mean	% died
37·5	90	0	—	—	100	70	23	25·0	0·74	20
35	—	0	Mould	—	—	—	29	17·9	0·50	3
32·5	—	0	Mould	—	—	—	30	15·5	0·34	0
30	—	28	17·8	0·19	0	—	26	18·0	0·81	10
27·5	—	25	21·0	0·10	17	—	29	22·7	0·19	3
25	—	29	27·7	0·14	0	—	30	28·9	0·40	0
22·5	—	27	36·2	0·57	10	—	26	43·0	0·63	13
20	—	21	81·6	1·41	20	—	27	79·4	0·34	10
17·5	—	0	—	—	100	—	0	—	—	100
35	0	7	41·6	1·15	77					
30	100	30	16·4	0·67	0					
25	50	35	34·1	0·36	8					
23·5	70	42	36·9	0·40	2					
37·5	30	13	31·6	3·50	57	10	0	—	—	100
35	—	32	23·9	0·28	6	—	12	40·3	0·92	59
32·5	—	30	23·5	0·40	3	—	24	36·4	1·53	17
30	—	30	25·1	0·36	7	—	25	34·9	0·42	17
27·5	—	30	31·0	0·50	0	—	25	38·5	0·83	17
25	—	33	35·6	0·36	0	—	39	47·8	0·47	8
22·5	—	30	57·3	0·71	0	—	41	64·3	0·60	27
20	—	21	98·3	2·41	23	—	0	—	—	100
17·5	—	0	—	—	100	—	0	—	—	100

A number of authors have grown larvae on several food materials. Good (1933) recorded a larval period of 31 days for middlings, 45·7 days for bran, 64·6 days for oatmeal and 89 days for white flour at 27° C. Cotton, Frankenfeld & Bayfield (1945), using a series of milling stocks, obtained much smaller differences at 80° F. (26·7° C.) with a lowest mean of 29·1 days on crushed whole wheat and a highest of 40·8 days on patent flour, their figures for bran being about 33 days. Miller (1944) compared six foods taken from various parts of the mill stream at 30° C., 75% R.H.

Four of these, crushed wheat, two pollards and a flour sample were fairly similar (18·6–20·7 days), but the two remaining white flour samples were much less satisfactory foods, the larva needing 24·8 and 35·1 days to pupation. Magis (1954), who prepared wholemeal flours from sixteen samples of grains, recorded at 27° C., 70% R.H. a mean larval period between 24 and 28 days for eleven samples of wheat, barley and oat flours, but 35 days for maizemeal and 40·5 for a polished-rice flour.

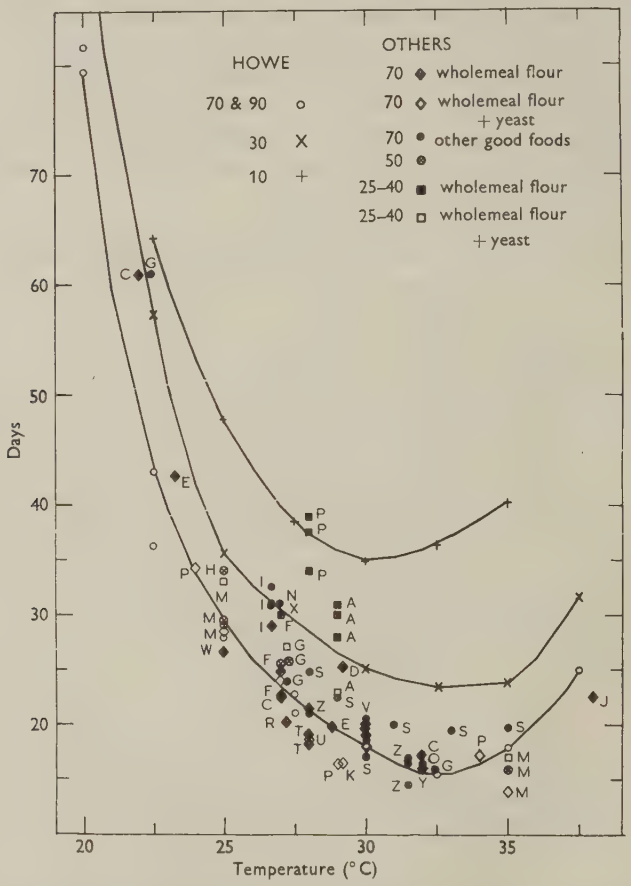

Fig. 2. The effect of temperature and relative humidity on the duration of the larval stage of *Tribolium confusum* fed on favourable foodstuffs. The lines are fitted to the results of the present work. The bottom line is for 70% R.H., the middle line for 30% R.H. and the top line for 10% R.H. Author abbreviations as Fig. 1 with, in addition, I = Cotton *et al.* (1945); J = Oosthuizen (1935); R = Bernard & Lemonde (1949); T = Lemonde & Bernard (1951*a*, *b*); W = Fraenkel & Blewett (1941); Y = Street & Palmer (1935); Z = Sweetman & Palmer (1928). The unlabelled points at 27° C. and 70% R.H. are from Magis (1954) and those at 30° C. and 70% R.H. from the six papers cited in the text.

The flours prepared from a primitive wheat and from pearl-barley were intermediate between these groups, but with the millet flour only one pupa was obtained from twenty larvae. Khalifa & Badawy (1955*a*) also showed that wheat flour was better than maizemeal which in turn was better than flour from polished rice. In addition, they showed that wheat bran was a little better than whole wheat flour. Mickel & Standish (1946) reported that soya products are poor foods especially at low humidities and Lipke *et al.* (1954) showed this to be due to a poisonous material in the soy bean.

In Fig. 2, the deleterious effect of low humidity in the present work is clearly shown. The results of Holdaway (1932) at 25, 50 and 75% R.H. at 27° C. are very similar, but Gray (1948) at 81° F. (27·2° C.) and Mickel & Standish (1946) at 25 and 35° C. failed to show such a marked effect of humidity, especially the latter at 35° C. Failure to adjust the moisture content of the food sufficiently before the start of the experiment could produce such a result.

Many early workers assumed that *T. confusum* always had six moults (e.g. Chapman, 1924) but Good (1936) showed that this number is increased by unfavourable conditions. Khalifa & Badawy (1955*a*) showed that the number varied between five and eight in favourable conditions, six being typical in the finer flours and seven being typical on crushed wheat or maize. On patent flour Park (1935) found seven or eight instars were usual.

The duration of the pupal period

The length of the pupal period was not affected by humidity. The effect of temperature on this period is shown in Table 3, together with a record of the mortality observed in these experiments. The length of the pupal period is also shown in

Table 3. *The duration of the pupal period in days at various temperatures, with details of mortality and sex ratio*

Temp. (° C.)	No.	Period (days)	No. of deaths	Sex ratio of adults ♀	♂
37·5	36	4·9	—	25	12
35	133	5·0	1 at 10%	72	62
32·5	112	5·5	—	55	59
30	174	6·1	1 at 90%	92	93
27·5	206	7·7	—	102	105
25	264	10·3	—	136	129
22·5	258	14·0	1 at 30%	145	113
—	—	—	1 at 70%	—	—
20	66	21·6	1 at 30%	42	31

Fig. 1 with data taken from the literature. A reasonably smooth curve can be drawn subjectively through the data of Table 3, but some of the published data included in Fig 1 are not consistent with this curve, in particular the records at 27° C. The values given by Estácio (1953) for 23·3 C. and Stanley (1946) for 28° C. can probably be discounted. Estácio apparently obtained this value in a laboratory room in which

the temperature was not rigorously controlled. The temperature quoted was the mean for the whole period of his experiment and it can be deduced that the temperature rose during this time. For both *T. confusum* and *T. castaneum* the values he quotes for the egg period are larger than are consistent with the results of other workers and the values for the pupal period much smaller. Stanley's (1946) value for 28° C. is puzzling because it is inconsistent with the other two original values given in his paper (29 and 31° C. in Fig 1) although all were based on the large number of insects.

T. confusum seldom dies in the pupal stage (Table 3) at any humidity and temperature combination in which larvae can grow. The most rapid completion of the stage is observed at the higher temperatures (Fig. 1). The food on which the

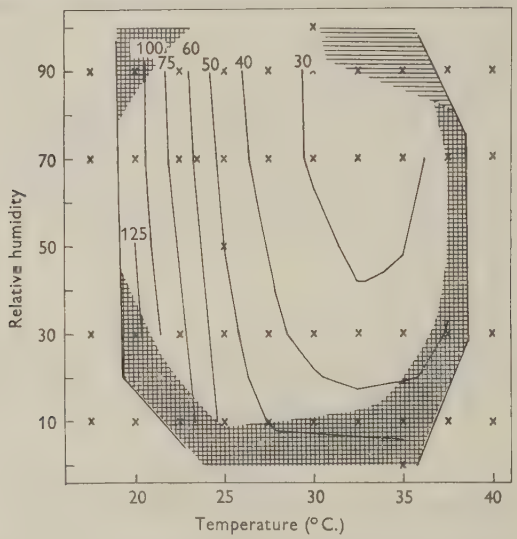

Fig. 3. The periods in days required for complete development from egg-laying to the formation of the adult of *Tribolium confusum* at various combinations of temperature and relative humidity. Crosses mark the temperature–humidity combinations for which results were obtained to determine the positions of the lines. The lines join all conditions at which development was completed in the numbers of days stated or beyond which development was not completed. The shaded area shows where developmental mortality exceeded 20 %.

larva feeds seldom has any obvious effect on the duration of the pupal period but Schneider (1943), who bred larvae at 29° C. on samples of white flour to which he added various levels of vitamin B, noted a lengthening of the pupal period by over half a day at the highest level of fortification. Park (1935) recorded means of 7·5 and 8·6 days at 28° C. for pupae bred respectively on fresh and heavily insect-conditioned white flour. Variability was much increased by the conditioning of the flour and Park obtained a maximum pupal period of 13 days. Both Park (70 %) and Schneider (46 %) noted heavy larval mortality on the foods which prolonged the pupal period.

The sex ratio of the pupae bred at each temperature is given in Table 3. At the upper and lower ends of the range more females were obtained but from 25 to 32·5° C. the sex ratio was very close to unity.

The total developmental period

Fig. 3 shows the period required for the complete development of *T. confusum* from the laying of the egg to the emergence of the adult when the larva feeds on wheatfeed. The optimum for rapid development lies close to 32·5° C. at 70% R.H. and above, the life cycle occupying about 25 days. Between 30 and 35° C. the life cycle can be completed in 30 days with little mortality at relative humidities down to 30%. Above 35° C., mortality exceeded 20% at all humidities and exceeded 60% below 50% R.H. The upper limit is just above 37·5° C., except at the high and low humidities where it is below 37·5° C. Mortalities of 15–25% were recorded (Fig. 3) in most of the experiments at low temperature or low humidity. The lower limit for complete development is between 17·5 and 20° C. except at low humidity where it rises above 20° C.

Discussion

Since *Tribolium confusum* and *T. castaneum* have been studied by the same means in this paper and an earlier one (Howe, 1956), a fair comparison of the two species can be made. The minimum and maximum temperatures for the hatching of the egg and the development of the larva, and the optimum for the rate of development of the larva are all about 2·5° C. lower for *T. confusum* than for *T. castaneum*. In neither species is mortality heavy during development except at the limiting high and low temperatures and low humidities.

Every stage of *T. confusum* develops more slowly than the corresponding stage of *T. castaneum* in the conditions optimal for each species. Thus the shortest egg periods are 2·6 days for *T. castaneum* and 3·9 days for *T. confusum*; the shortest larval periods at 70% R.H. are 12·9 and 15·5 days, respectively, and the shortest pupal periods are 3·9 and 4·9 days. This relationship pertains throughout a temperature scale in which the optima serve as the origin (see Fig. 5); for instance, at 15° C. below the optimum, *T. castaneum* still grows faster in all stages than *T. confusum*. On the natural temperature scale, however, *T. confusum* develops the faster in the lower part of the range.

There is sufficient information available on *T. confusum* to examine the effect of temperature on the rate of development of the various stages. In Fig. 4 the reciprocals of the egg period data of Fig. 1 are plotted against temperature and a line drawn by eye through the points. The deleterious effect of high temperature is shown by the arching of the curve to an optimum of about 35° C. A straight line can reasonably be fitted to the region between 22·5 and 32·5° C., but clearly below 22·5° C. the line curves. Butler (1949) plotted a straight line through some of the published data and by extrapolating the line predicted a developmental threshold of 15° C. The curvature observed in Fig. 4 would lead to a lower developmental minimum,

whereas in fact the observed developmental minimum is higher—just under 17° C. Lin *et al.* (1954) attributed the failure of embryonic development below 17° C. to the exhaustion of the food reserves in the egg. Estácio (1953) used the Blunck–Bodenheimer hyperbola formula to calculate the developmental minima of the various stages from his own experimental observations. He calculated a very low developmental minimum for the pupal stage, because, as has already been mentioned, he appears to have underestimated the temperature for the lower of the two conditions he used. There seems to be little point in calculating this theoretical value except as a very rough guide to the limiting low temperature.

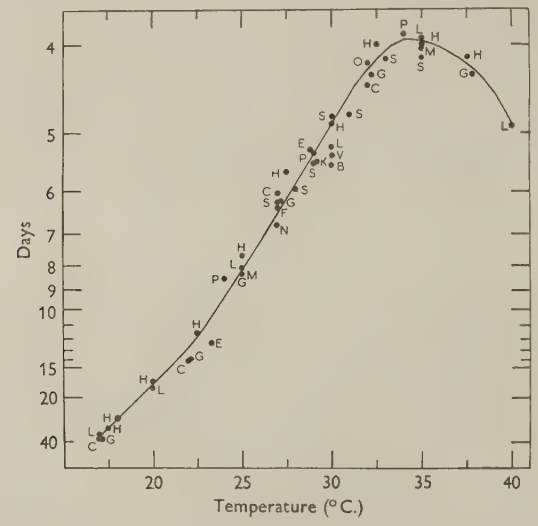

Fig. 4. The effect of temperature on the rate of development of the egg of *Tribolium confusum* measured as the reciprocal of the duration in days of this stage. The line is drawn to correspond exactly with that shown in Fig. 1. Author abbreviations as in Fig. 1.

However, curves relating the rate of development (i.e. the reciprocal of the developmental period) to temperature and humidity may be useful for comparing the two species or the effects of environmental conditions because the developmental rate is directly related to the intrinsic rate of increase of the species. In Fig. 5 the rates of development of the eggs and the pupae of *T. confusum* and *T. castaneum* are plotted. The lines are drawn to correspond exactly with the lines for developmental period given in Fig. 1 of this paper and Fig. 1 of Howe (1956). In Fig. 6 the rates of larval development are given for the two species at different relative humidities, the lines again being drawn through values corresponding to the reciprocals of the values plotted in Figs. 2 of each paper. In Fig. 5, the optimum temperature is used as an approximate origin; in Fig. 6, the normal Centigrade scale is used. The rate curves for eggs and pupae of both species (Fig. 5) show the sigmoid shape with curvature at the lower end of the range and the arching at

high temperatures already mentioned. The lower curvature does not appear in most of the curves for larvae (Fig. 6). In both species at 70% R.H. there is a well-marked straight-line relationship between temperature and developmental rate below the optimum. At lower humidities, the arching which is present at 70% R.H. close to the optimum temperature only, extends increasingly to lower temperatures, so that at 10% R.H. the data are represented entirely by this arc. From Fig. 6 it

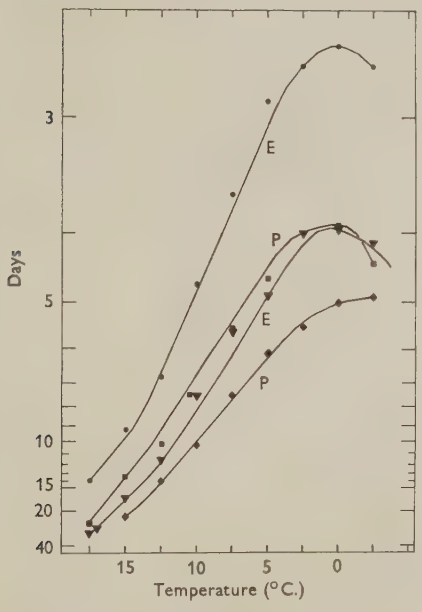

Fig. 5

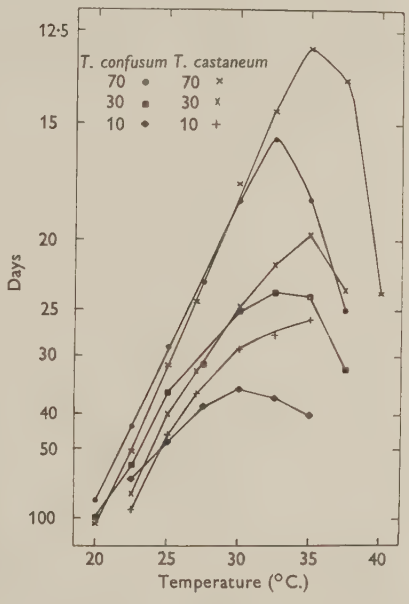

Fig. 6

Fig. 5. The effect of temperature on the rate of development of the eggs (E) and pupae (P) of *Tribolium confusum* and *T. castaneum*. The points marked are the original data presented in this paper and by Howe (1956); the lines correspond to those drawn in Fig. 1 of this paper and Howe (1956). The temperature scale is measured from the approximate optimum for each species, i.e. 37·5° C. for *T. castaneum*, and 35° C. for *T. confusum*. The lower lines are for *T. confusum*.

Fig. 6. The effect of temperature and relative humidity on the rate of development on wheat-feed of larvae of *Tribolium confusum* and *T. castaneum* using the lines drawn in Fig. 2 of this paper and Howe (1956), and plotting the original data of these papers.

can be deduced that the minimum developmental temperature threshold is probably highest at the lowest humidities for both species. It is clear from this figure that, as mentioned previously, at high temperatures *T. castaneum* completes the larval stage more quickly than *T. confusum* and at low temperatures more slowly. Even if experiments had not been performed at 20° C. it would have been possible to predict that *T. castaneum* had the higher developmental minimum. The larval stage

is approximately equal in length in the two species at about 24·5° C. at 10% R.H., and at about 29° C. at 30 and 70% R.H.

The periods required for the complete developmental cycle from oviposition to the formation of the adult are compared in Fig. 7. These are identical in the two species at about 23° C. at 90% R.H., at about 27° C. at 30% R.H. and about 24·5° C. at 10% R.H., i.e. at somewhat lower temperatures than for the larval stage alone. This is because the egg and pupal stages together are shorter in *T. castaneum* in all temperature and humidity combinations. Above 22·5° C., extremes of humidity apparently operate in favour of *T. castaneum*. In moderate humidities (30–70% R.H.)

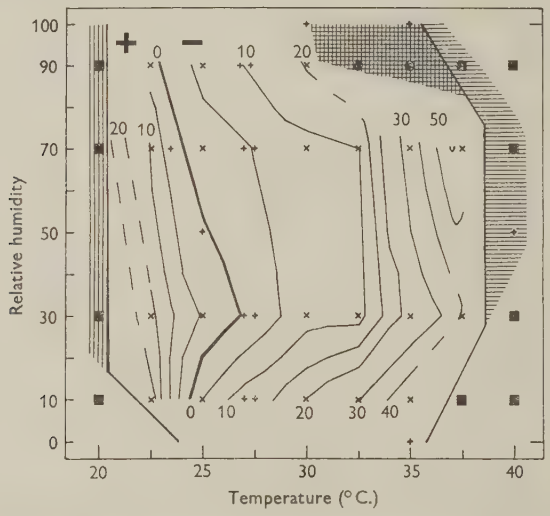

Fig. 7. A comparison of the rates of development from egg-laying to adult of *T. confusum* and *T. castaneum*. *T. confusum* develops most rapidly in the zone marked + and least rapidly in that marked −. The number against a line is the percentage increase in rate of development of the faster over the slower species represented by that line. Experimental data were obtained for both species at conditions marked × and for one species only at conditions marked +. Shaded areas represent conditions in which one species failed to grow, or was overcome by mould.

between 22·5 and 32·5° C., the rate of development curves in Figs. 4–6 are almost straight lines, consequently the ratios of growth rates plotted in Fig. 7 are widely spaced in this area.

As in *T. castaneum*, the female is on average heavier than the male and the heaviest individuals are produced at the higher humidities and at lower temperatures. The average weight of a *T. confusum* adult is about 0·1–0·4 mg. above that of a *T. castaneum* of the same sex grown in similar conditions.

Miss S. M. Nightingale assisted with the experimental work.

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INFECTIVITY OF APHIDS BRED ON VIRUS-INFECTED CAULIFLOWER PLANTS

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Caged cauliflower plants infected with either cabbage black ring spot virus (CBRSV) or cauliflower mosaic virus (ClMV) were colonized with *Myzus persicae* or *Brevicoryne brassicae*. Winged and wingless aphids that voluntarily flew or walked from these plants were transferred singly to healthy cauliflower or other brassica seedlings to compare their feeding behaviour and ability to transmit the viruses. Wingless aphids settled to probe more readily than winged, and *B. brassicae* was initially more restless than *M. persicae*. ClMV was more readily transmitted than CBRSV by both species, and *B. brassicae* rarely transmitted CBRSV. Wingless aphids transmitted less often than winged ones, and no wingless *B. brassicae* transmitted CBRSV, although they did ClMV. Fewer aphids transmitted CBRSV from old plants than from young ones, but plant age had little effect on ClMV transmission.

When insects are tested to see whether they transmit a virus, they are allowed to spend different periods of time on infected plants before being transferred to healthy ones. Fasted aphids are more likely to transmit non-persistent viruses after a brief acquisition feed than after a long one (Watson & Roberts, 1939). It has been postulated, therefore, that winged aphids (alatae) are more likely than wingless ones (apterae) to spread non-persistent viruses in crops, because apterae move less frequently and may have fed for a long period before leaving the infected plant (Bawden, 1948). Field experiments in Britain have supported this idea, as also have the correlations between virus spread with trap catches of winged aphids but not with counts of wingless ones on plants (Broadbent, 1950; Watson & Healy, 1953; Hollings, 1955). Apterae are thought to be relatively unimportant as vectors because when they carry viruses to neighbouring plants such plants are likely already to have been infected by the alatae which colonized them earlier (Broadbent, 1957).

The evidence on the frequency with which apterae move from plant to plant is conflicting (Broadbent, 1953), and recent data have not resolved the problem: Shands & Simpson (1959) assume from population data that apterae move frequently between young potato plants in Maine, U.S.A., but Fisken (1959) in Scotland concluded that apterae play little part in spreading the infestation through the crop. In hot climates, however, apterae move more frequently than in temperate ones, and are thought to be important as vectors of viruses (Bald, Norris & Helson, 1950).

Alatae may acquire non-persistent viruses when they probe infected plants during intervals between flights within crops, but apterae will normally only acquire and spread virus when they have developed on an infected plant. Results of laboratory

tests in which aphids are moved from infected to healthy plants may not apply to conditions in the field. Apterae are mostly used because they are usually more numerous and easier to handle. Feeding is often ended artificially by disturbing the aphids, and Hoggan (1933), Bradley (1952) and Sylvester (1954) all found that such disturbance during the acquisition feed gave fewer transmissions than when the aphids ended penetration voluntarily. Forced interruption of the test feed, however, did not affect the efficiency of transmission. Bradley (1954) found that *Myzus persicae* (Sulzer) were not infective with potato virus *Y* after a prolonged probe on infected tobacco, but sometimes became so when such a probe was followed by a short one. Kennedy, Booth & Kershaw (1959) found that 70% of *Aphis fabae* Scop. that made prolonged probes on bean leaves paused to make a brief one before flying, so aphids that develop on infected plants could become infective by making short probes before departure.

Single apterous and alate aphids have rarely been compared as vectors experimentally in adequate numbers. However, apterae of *A. ferruginea-striata* Essig were the more efficient in transmitting poison hemlock ringspot virus (Freitag & Severin, 1945*b*); also apterae of *M. persicae* with lettuce mosaic virus (Sylvester, 1955); and of *M. persicae* with cabbage black ringspot virus, although the differences were not statistically significant (Hamlyn, 1953). By contrast, alatae of *Rhopalosiphum conii* (Dvd.) were the more efficient in transmitting poison hemlock ringspot virus and celery yellow spot virus (Freitag & Severin, 1945*b*, *a*). Storey & Ryland (1955) found that in some races of *Aphis craccivora* (Koch) alatae were better vectors than apterae of groundnut rosette virus, whereas in other races the apterae were best. In still other races winged and wingless behaved similarly.

The experiments described below were designed to find the proportion of the winged and wingless aphids developed on infected plants that were infective, as well as the ease with which they could obtain virus from plants of different ages.

Methods

The aphids used were *M. persicae* and *Brevicoryne brassicae* (L.) collected from cauliflower plants (*Brassica oleracea* L. var. *botrytis* L., variety Extra Early Roscoff) infected either with cabbage black ringspot virus (CBRSV) which is non-persistent, or with cauliflower mosaic virus (ClMV) which might be called semi-persistent because the response to preliminary fasting before the infection feed is small or non-existent, and virus may persist for several hours in *M. persicae* and for at least a day in *B. brassicae* (Hamlyn, 1953, 1955; van Hoof, 1954).

Preliminary results and a photograph of the cages were published earlier (Broadbent, 1957). The plants were grown in the ground out of doors and each was later covered with a muslin and transparent 'xylonite' cage, measuring 26 × 26 × 24 in., which fitted on to a wooden base placed round the stem of the plant. The cages were painted white and aphids could be seen easily when crawling on any part of them; they were placed in position after the plants had been infested with aphids from healthy pe-tsai (*Brassica pekinensis* Rupr.) plants grown under glass. Aphid

colonies were left to develop for at least 3 weeks before the tests began. Although the cages partially shaded the plants, growth was similar to that of field plants, and the behaviour of aphids on them is likely to have approximated more to that of a 'wild' population than to behaviour under glass.

Before a period of testing, the cages were cleared of aphids, leaving only those actually on the plants. Care was taken to prevent large plants from touching the cages. Then those aphids which flew or walked from the plants were collected in a glass tube by suction at 30 min. intervals. Few of the infective aphids were likely to have lost their infectivity within this time, even had they left the plants as soon as the cages were replaced.

The aphids collected were placed individually on healthy cauliflower, occasionally on turnip (*B. rapa* L.) or pe-tsai seedlings, under wide glass tubes with nylon gauze covers. The seedlings were shaded for an hour to encourage the aphids to settle and feed. If they left the seedlings within 10 min. they were replaced.

A preliminary test showed that seedlings of the different species were equally suitable. Apterous aphids, after fasting for 3–4 hr., were allowed to probe turnips infected with ClMV for 5 min. and were then transferred in twos to seedlings, the following numbers of which were infected (each out of thirty tested):

Myzus persicae: 10 cauliflower, 11 turnip, 11 pe-tsai.
Brevicoryne brassicae: 8 cauliflower, 6 turnip, 14 pe-tsai.

The same tests were used to see whether the proportion of transmissions differed with different operators. The percentages of transmissions obtained by four people were: 46, 23, 35 and 30. Most of the experiments were done, therefore, by the first person, a few by the third.

After about 24 hr. the aphids were examined to see whether they were alive or dead, and whether they were on the plants; evidence of having spent some time on the seedlings was obtained also by noting the presence of nymphs and, with *B. brassicae* which has toxic saliva, damaged leaves. Surviving aphids were then killed and the plants kept in an insect-proof glasshouse for 4 or 5 weeks to ensure that infected plants would develop symptoms.

Experiments were done as opportunity occurred during the months May to October, 1953–8. The results, therefore, cannot be easily analysed statistically because apterae and alatae from the same plant could rarely be directly compared, and growing conditions varied from one period to another. Weather would not only affect the plant, but also probably the virus concentration and its availability to the aphids, as well as the activity and efficiency of the vectors. Nevertheless, as the figures in Table 1 summarize several tests with different batches of aphids from different plants, any large differences probably represent real effects. An analysis of variance on the data was kindly done by Mr D. Cooke, Glasshouse Crops Research Institute.

Results and discussion

When apterous aphids left a plant they walked down the stem on to the floor of the cage. Most alatae flew to the top of the cage, or to the side towards the brightest light, but some dropped on to the floor and later flew up. During several periods when they were watched 49% flew to the top, 20% to the sides and 31% dropped to the floor.

The aphids usually settled to probe quickly, but *B. brassicae* was initially more restless than *M. persicae*: about 2% apterous and 4% alate *M. persicae* were replaced on the seedlings after 10 min., in contrast to 7% apterous and 16% alate *B. brassicae*. 24 hr. later also, more *B. brassicae* (20% apterae, 22% alatae) were found off the seedlings than *M. persicae* (6% apterae, 9% alatae). Most of these left no evidence of feeding, but they were included when calculating the proportions of aphids that transmitted virus because they could have done this during short probes while wandering on the plants. These results confirmed earlier experiments which showed that the young alate *B. brassicae* were more active than *M. persicae* (Broadbent, 1949).

TABLE 1. *Numbers of aphids tested and percentage that transmitted virus*

Cauliflower plants (virus source)		Cabbage black ringspot virus				Cauliflower mosaic virus			
		M. persicae		*B. brassicae*		*M. persicae*		*B. brassicae*	
		Apt.	Alate	Apt.	Alate	Apt.	Alate	Apt.	Alate
Young	No.	47	39	7	234	50	95	40	291
	%	*12·8*	*5·1*	*0*	*5·1*	*12·0*	*15·8*	*2·5*	*16·5*
Medium	No.	30	77	46	91	118	85	23	110
	%	*10·0*	*19·5*	*0*	*1·1*	*22·0*	*18·8*	*8·7*	*22·7*
Old	No.	68	84	43	140	92	116	36	127
	%	*2·9*	*6·0*	*0*	*2·1*	*17·4*	*22·4*	*27·8*	*8·7*
Total	No	145	200	96	465	260	296	99	528
	%	*7·6*	*11·0*	*0*	*3·4*	*18·5*	*19·3*	*13·1*	*15·9*

About 6% apterous and 10% alate *B. brassicae*, and 4% apterous and 5% alate *M. persicae*, died as a result of handling, indicating that *B. brassicae* is the more fragile aphid. About half of these left no evidence of feeding, and as they might have died without even probing, they were omitted from the calculations.

Table 1 shows the numbers of aphids tested, apart from those just mentioned, and the proportions that transmitted virus. Between one in five and one in twenty of the winged aphids that leave an infected plant might be expected to infect healthy susceptible plants provided they land on them soon after leaving the infected one. ClMV was more readily transmitted by both species of aphids than CBRSV (differences statistically significant at the 5% level). The differences between the aphid species were significant also. *B. brassicae* was a particularly poor vector of CBRSV; this confirms the results of Kvicala (1948) who found *M. persicae* to be a more efficient vector of CBRSV than *B. brassicae*. *B. brassicae* was slightly less efficient than *M. persicae* as a vector of ClMV, possibly because more *B. brassicae*

moved off the seedlings. This contrasts with the findings of Severin & Tompkins (1948) and van Hoof (1954) that *B. brassicae* infected more cauliflower plants with ClMV than did *M. persicae* in laboratory tests.

Although apterae of both species settled on the seedlings more readily than alatae, they infected fewer plants, but the differences are not statistically significant. In particular, apterous *B. brassicae* did not transmit CBRSV, although they did ClMV. The alatae were mostly newly matured and not known to have flown previously; possibly such aphids feed only intermittently during the period between final ecdysis and flight, whereas apterae may have fed for longer periods before moving and would therefore be poorer vectors of a non-persistent virus such as CBRSV. Alternatively, apterae could make brief probes and acquire virus while crawling round the plant, but many might lose their infectivity with CBRSV during the time they took to walk down the stem and on to the cage. Shading the seedlings for an hour after the aphids had been placed on them might have been a mistake, for the alatae, which settled less readily than the apterae, were the more efficient vectors, and in tests with potato virus *Y*, Bradley & Rideout (1953) obtained fewer infections with long (2 min.) than with short (15 sec. to 1 min.) initial test-feeding punctures.

Aphids retain ClMV for longer than CBRSV (Hamlyn, 1953, 1955; van Hoof, 1954) and have less opportunity of acquiring CBRSV because it occurs mainly in the older lower leaves of brassica crop plants, and even there is localized in parts that show symptoms. ClMV occurs in high concentration in all new leaves produced by infected plants (Broadbent, 1954). Only young infected cauliflower plants contain much CBRSV in the young upper leaves, and in the present experiments fewer *M. persicae* transmitted CBRSV from old plants (4·6%) than from young and medium aged ones (13·5%), whereas 20·2% transmitted ClMV from old plants and 18·1% from young and medium ones; *B. brassicae* transmitted CBRSV from 1·2% of old and medium plants and 5·0% of young ones, but ClMV from 12·9% of old plants and 16·4% of young and medium ones. A χ^2 test on the CBRSV results showed that the differences were significant at the 5% level, but whereas the old plants were poorer sources than medium and young ones for *M. persicae*, the old and medium plants were poorer than the young for *B. brassicae*.

The proportions of plants infected by *M. persicae* are similar to those to be expected in glasshouse tests with single aphids and the usual methods, but with *B. brassicae* and CBRSV the proportion is much less. It is unwise, therefore, to draw conclusions from laboratory studies about the relative importance of different species as vectors of a virus in the field. It must also be remembered that experimental comparisons between different vector species may apply only to the individual aphid races and virus strains tested. Most viruses occur in several strains, some of which differ in their relationship to insect vectors, and Stubbs (1955), Storey & Ryland (1955) and Björling & Ossiannilsson (1958) showed that great differences in ability to transmit a virus exist within an aphid species.

A major obstacle in applying the results of these experiments to the field is that, although the aphids left the infected plants of their own accord, they were then transferred by hand to the test plants. Several instances have been quoted where

handling decreased the number of transmissions because the aphids were disturbed during their acquisition feed, but there is no evidence that handling is detrimental after this feed has been terminated naturally. The results suggest that apterae might be nearly as efficient as alatae in transmitting ClMV and CBRSV when they move of their own accord from infected to healthy plants. However, they move from one plant to another relatively infrequently in the field and experiments there indicated that most spread of these viruses was by alatae (Broadbent, 1957). Although *B. brassicae* was somewhat less efficient in these tests than *M. persicae*, severe outbreaks of ClMV are usually associated with large numbers of *B. brassicae*, which become more numerous than *M. persicae* in favourable ecological conditions.

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APHID BEHAVIOUR ON HEALTHY AND ON YELLOWS-VIRUS-INFECTED SUGAR BEET

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(With 1 Text-figure)

Myzus persicae Sulz., *M. ascalonicus* Doncaster, *Aphis fabae* Scop. and *Aulacorthum solani* Kalt., when caged on sugar-beet leaves in the glasshouse preferred yellows-virus-infected leaves to healthy ones; they chose those with the most severe symptoms on which they bred more rapidly and lived longer than on green leaves. *M. persicae* behaved similarly on whole plants in the glasshouse. The previous host influenced the aphid's preference. Differences between the multiplication rates of aphids on healthy plants of inbred sugar-beet varieties were eliminated or reversed by infection with yellows virus.

Spraying healthy plants with either sugar solutions or hydrolysate of casein increased the multiplication rate of *M. persicae*, but much less than did virus infection.

Aphis fabae Scop. is a common pest of sugar beet, and *Myzus persicae* Sulz. the most important field vector of sugar-beet yellows virus. Kennedy, Ibbotson & Booth (1950) showed that multiplication of *A. fabae* on healthy plants depends on the physiological age of the leaf; and, in general, aphids prefer young or senescing leaves to fully functional, mature ones. Hijner & Cordon (1953) found that *M. persicae* bred more rapidly on sugar beet infected with virus yellows than on healthy ones and, when given a choice, preferred those leaves exhibiting the strongest yellows symptoms. Experiments in the glasshouse at Dunholme during the summers of 1957 and 1958 confirmed and extended these results. Virus infection was found to make leaves of any age favourable for aphid colonization and the results illustrate the complexity of the host–parasite relationship shown by aphids.

Methods and materials

M. persicae cultured on turnip were caged on the underside of sugar-beet leaves, in nylon-covered cells made by boring $\frac{1}{2}$ in. diameter holes in $\frac{1}{8}$ in. thick balsa wood. Each cell was held in place by metal clips against a similarly drilled piece of $\frac{1}{16}$ in. balsa wood on the other side of the leaf. To determine feeding preferences two equal areas of leaf were enclosed in an oval cage (length 1 in.) constructed from $\frac{1}{8}$ in. balsa wood clipped against a solid piece of $\frac{1}{16}$ in. balsa backing. In some experiments whole leaves of young plants were enclosed in rectangular cages made of balsa strip, cellophane and nylon. In all preference experiments the cages were kept horizontal and in uniform lighting because *M. persicae* are attracted by light.

The sugar beet were grown in pots in the glasshouse. Two isolates of beet yellows virus were used:

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V.Y.50, which gives very severe vein etch, distortion and stunting. It produces symptoms on seedlings in 7–10 days and sap from infected plants often precipitates specifically with virus antiserum at a dilution of 1:128.

V.Y.4, which gives only a faint and ephemeral vein etch. Symptoms develop in 4–6 weeks and the maximum precipitation titre of the sap is 1:4.

The sugar beet used were of five inbred varieties selected for varying susceptibility to yellows. Their reactions to V.Y.50 are:

M9 }
1/201 } very susceptible,

5/214 moderately susceptible,
311/2 gives yellowing but only faint vein etch,
41/675 tolerant.

Feeding preferences

The distribution of *M. persicae* on mature leaves of the same age when left to choose between healthy leaves and those infected with Y.V.4 or V.Y.50, was determined by placing in each preference cage ten to forty aphids, the number depending on their age and size. After a day each cage was opened and the number of aphids (young progeny excepted) on the cage and on different sections of the leaf was noted. All insects were removed and killed, and the cage reloaded with fresh aphids.

Table 1. *Distribution of* Myzus persicae *on mature leaves of healthy and yellows-infected sugar beet*

No. of experiments	Total no. of aphids used	Age of aphids	Average percentage of aphids on		
			Healthy	V.Y.50	Cage
21	227	Adults	29	54	17
12	106	Nymphs	22	50	28
			Healthy	V.Y.4	Cage
27	288	Adults	21	55	25
			V.Y.4	V.Y.50	Cage
21	216	Adults	34	40	26
7	98	Nymphs	33	48	18
			Healthy	Healthy	Cage
15	112	Adults	40	37	23
			V.Y.4	V.Y.4	Cage
12	287	Adults and nymphs	43	38	19
			V.Y.50	V.Y.50	Cage
12	177	Adults and nymphs	42	41	18

Table 1 shows that both adults and nymphs prefer virus infected to healthy leaves and those infected with V.Y.50 to those with V.Y.4.

The aphid's choice is influenced by the leaf age (Table 2). Adult and nymphal aphids prefer young to mature healthy leaves, but they prefer mature infected leaves to young healthy ones, especially when the infected leaves show severe symptoms. The severity of the symptoms also determined the preference for young or old

leaves on an infected plant; aphids prefer the young to the mature leaves of a recently infected old plant, but they prefer the mature yellow leaves to the symptomless young ones of plants infected for some weeks.

TABLE 2. *Proportion of* Myzus persicae (*adults and nymphs*) *on old and young leaves*

No. of experiments	Total no. of aphids used	Average percentage of aphids on different types of leaves		
		Healthy young	Healthy mature	Cage
8	209	60	28	12
		Healthy young	V.Y. 50 mature	Cage
15	275	36	54	10
		V.Y. 50 young	Healthy mature	Cage
15	191	65	26	9

Effect of previous feeding on preference

The aphids' previous host plant appears to influence their preference (Table 3). Aphids cultured for longer than a week on turnip, healthy sugar beet or beet infected with V.Y. 50 were given the choice of feeding on healthy or V.Y. 50-infected leaves of sugar beet. The greatest preference for V.Y. 50 was shown by those bred on beet infected with yellows virus and least by those bred on healthy beet.

TABLE 3. *Feeding preferences of adult* Myzus persicae *cultured on different plants*

No. of experiments	Total no. of aphids used	Previous host plant	Average percentage of aphids on different types of leaves		
			Healthy	V.Y. 50	Cage
41	395	Turnip	29	54	17
15	224	Healthy sugar beet	41	43	16
21	403	Sugar beet with V.Y. 50	28	60	12

MULTIPLICATION OF *MYZUS PERSICAE* ON YELLOWS-INFECTED AND HEALTHY INBRED LINES OF SUGAR BEET

In three separate experiments, each carried out in duplicate, aphids were caged on leaves of similar age of healthy, V.Y. 4 and V.Y. 50 infected plants of each of the inbred lines. Five recently moulted adults or late penultimate instar nymphs were placed in each cage and the surviving adults and their progeny were counted daily. After counting, the progeny were removed and killed. Table 4 shows the number of young produced per adult per day—a value obtained by dividing each day's count of young by the number of live adults in the cage that day, and averaging each day's figures obtained during the course of a series of weekly experiments. When plotting cumulatively the young/adult/day for the different treatments (Fig. 1 *b*) the most consistent daily results were later found to be obtained by plotting the number of young produced per adult alive on the previous day. The graph clearly shows that multiplication rate of *M. persicae* was correlated with the severity of the symptoms. Aphid multiplication was three times greater on the healthy

plants of line 41/675 than on line 1/201, but this difference was eliminated or reversed by yellows infection, which greatly increased the multiplication rates on both lines. The difference between multiplication rate on young, mature and old leaves of yellows-infected plants is far less than on healthy ones; but, in general, the reproductive rate is greater on young leaves than on mature ones, both of healthy plants and those infected with either V.Y.4 or V.Y.50 (Table 5).

There are two possible explanations of these results. Either young leaves contain something that stimulates aphid multiplication and its concentration falls as the

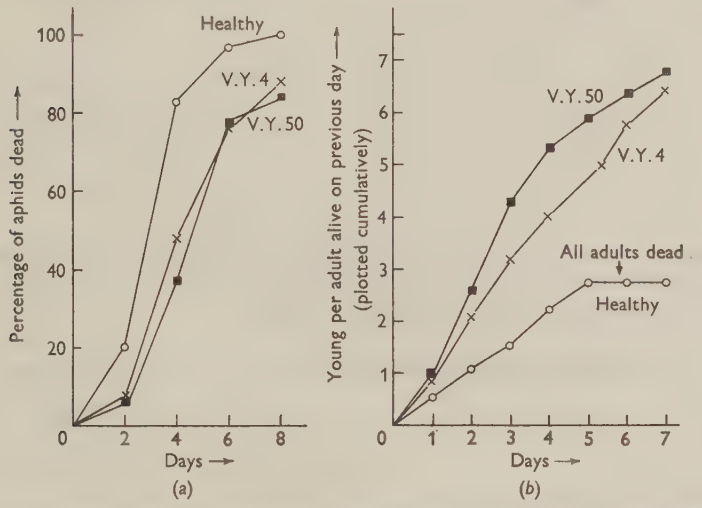

Fig. 1. (*a*) Life span, and (*b*) multiplication rate of caged *Myzus persicae* Sulz. on mature leaves of healthy and yellows-infected sugar beet.

TABLE 4. *Multiplication of* Myzus persicae *on inbred lines of yellows-infected and healthy sugar beet*

	Young/adult/day*		
Inbred line	Healthy	V.Y.4	V.Y.50
1/201	0·18	0·93	1·49
41/675	0·58	1·3	1·99
5/214	0·48	1·06	1·14
Average	0·41	1·1	1·54
	Total young†		
1/201	15	125	135
41/675	47	120	189
5/214	39	99	108
Average	34	115	144

* Figures based on average of six separate experiments.
† Figures refer to sum of six experiments.

leaves mature, or, an inhibitor of aphid growth is produced in older leaves. Virus infection may affect aphid multiplication either by increasing the concentration of the stimulant or decreasing that of the inhibitor.

TABLE 5. *Multiplication of* Myzus persicae *on yellows-infected and healthy sugar beet leaves of different ages*

Treatment	Young/adult/day: Young leaves	Mature leaves	Old leaves
Healthy	1·02	0·4	0·21
V.Y.4	1·31	1·12	1·09
V.Y.50	1·48	1·51	0·91
	Total young		
Healthy	82	101	10
V.Y.4	79	344	85
V.Y.50	91	432	37

LENGTH OF LIFE OF APHIDS

M. persicae die sooner on mature or senescent leaves of healthy plants than on corresponding ones with yellows; but they live equally long on young leaves of infected and uninfected plants (Table 6 and Fig. 1 *a*).

TABLE 6. *Rate at which caged* Myzus persicae *die on sugar beet leaves*

	Days after loading cages: 2	4	6	8
	Percentage of aphids dead			
Mature leaves (60 aphids per treatment)				
Healthy	20	83	97	100
V.Y.4	8	48	77	88
V.Y.50	7	37	78	84
Young leaves (20 aphids per treatment)				
Healthy	15	50	80	95
V.Y.4	20	55	70	90
V.Y.50	15	60	85	90
Old leaves (20 aphids per treatment)				
Healthy	35	90	100	100
V.Y.4	25	60	70	80
V.Y.50	0	53	93	100

FEEDING PREFERENCES, RATE OF REPRODUCTION AND LIFE SPAN OF OTHER APHID SPECIES

The experiments described in the previous sections were repeated with *M. ascalonicus* Doncaster, *Aphis fabae* Scop. and *Aulacorthum solani* Kalt. all of which infest sugar beet and can transmit yellows virus. Each species preferred to feed on leaves of yellows-infected plants to healthy ones and when doing so produced more young and lived longer (Tables 7 and 8).

TABLE 7. *Feeding preferences and reproduction rate of various aphid species on healthy and yellows-infected sugar beet*

	Average percentage of aphids		
	Healthy	V.Y.50	Cage
Preference*			
Myzus persicae Sulz.	29	54	17
M. ascalonicus Doncaster	25	61	14
Aphis fabae Scop.	22	51	27
Aulacorthum solani Kalt.	14	77	9
	Young/adult/day		
Reproduction	Healthy	V.Y.4	V.Y.50
Myzus persicae Sulz.	0·4	1·12	1·51
M. ascalonicus Doncaster	0·87	0·95	1·8
Aphis fabae Scop.	0·99	2·0	1·4
Aulacorthum solani Kalt.	0·17	0·55	0·87

* Based on at least ten separate experiments involving more than 100 aphids.

TABLE 8. *Length of life of various aphids on healthy and yellows-infected sugar beet*

	Days after loading cages			
	2	4	6	8
	Percentage of aphids dead			
Myzus ascalonicus				
Healthy	0	20	90	90
V.Y.4	10	10	20	20
V.Y.50	0	0	20	20
Aphis fabae				
Healthy	40	80	90	90
V.Y.4	20	40	40	40
V.Y.50	10	30	40	40
Aulacorthum solani				
Healthy	50	80	90	90
V.Y.4	0	0	0	0
V.Y.50	0	0	10	10

Ten aphids used for each treatment.

Distribution of Myzus persicae *on whole plants*

In three experiments *M. persicae* were left to reproduce and distribute themselves freely on plants grouped together in large insect-proof cages. Potted plants, with twelve to sixteen leaves, of inbred lines 1/201 and 5/214, healthy or infected with either V.Y.4 or V.Y.50, were each infested with twenty adult wingless *M. persicae*. Duplicate plants were arranged so that plants of different treatments were contiguous in the two cages. The aphids were counted every 10 days (Table 9). In another experiment, aphid-free plants of different inbred lines, some healthy and some infected, were arranged around an aphid-infested turnip. Ten days later the average number of adult and nymphal aphids per plant was respectively 16 and 32 on the healthy, 41 and 92 on the V.Y.4 and 50 and 127 on the V.Y.50. On average over

these four experiments, the young per adult ratio was 2·2 on healthy, and 2·96 on V.Y.4 and 2·91 on V.Y.50. But it is more interesting to compare the total number of aphids on healthy and infected plants. The ratios correspond closely with those from leaf-cage experiments (Table 10). This agreement suggests that the leaf cages used did not unduly affect the behaviour of *M. persicae*. In the experiments with whole plants, yellows spread to all the initially healthy plants. The symptoms were mostly mild. After 6 weeks aphids were distributed more uniformly, but even then V.Y.50 plants still had the largest colonies (Tables 9 and 10).

TABLE 9. *Distribution of* Myzus persicae *on contiguous sugar beet in a large cage*

	Type of plant and leaf					
	Healthy		V.Y.4		V.Y.50	
	Young	Adult	Young	Adult	Young	Adult
	Numbers of adults and nymphs					
After 10 days						
Variety 1/201	42	93	89	299	118	371
Variety 5/214	56	130	150	466	159	506
	Total number of aphids					
After 40 days						
Variety 1/201	250		320		350	
Variety 5/214	405		420		612	

TABLE 10. *Comparison of results obtained in small leaf cages and on entire plants*

	Ratios determined from			
	Small leaf cages		Whole plants	
	Young/adult/day	Total young	Total aphids after 10 days	Total aphids after 40 days
V.Y.4 : healthy	2·8	3·4	3·0	1·13
V.Y.50 : healthy	3·8	4·3	3·7	1·5
V.Y.4 : V.Y.50	1·4	1·3	1·2	1·3

Effect on aphid multiplication of spraying leaves with chemicals

Young, healthy leaves and virus infected ones contain more reducing sugars than mature green leaves (Watson, 1955) and, by analogy with Fife's (1956) results on curly top disease, possibly also more amino acids. A few experiments were made to see whether the multiplication rate was affected by spraying plants with solutions of glucose, sucrose and hydrolysate of casein. The reproductive rate was slightly increased by all the solutions, but this effect was small in comparison to that produced by infection with yellows virus.

I thank Dr R. Hull for suggesting the problem and Miss E. Marshall for valuable practical assistance.

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STUDIES UPON THE COPPER FUNGICIDES

XI. ABSORPTION OF SOLUBLE COPPER IN RELATION TO STAGE OF LEAF GROWTH AND TO LEAF INJURY

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(With 1 Text-figure)

A study of the absorption of soluble copper by apple leaves was undertaken with a view to obtaining a better understanding of the mechanism of copper spray damage.

The ability of young apple leaves to absorb copper was found to be relatively high and this may be due to the large number of stomata and hairs present. As the leaves grew more mature this capacity fell, rose again to a maximum in July or August and then fell again as the leaves approached senescence. It is suggested that these changes may be due to seasonal changes in leaf metabolism, which probably affects nutrient absorption by leaves as in roots.

It has been observed in the field that apple leaves are often more susceptible to copper spray damage in the autumn. Since absorption is then at a low level it is concluded that the rate of absorption is not the only factor affecting damage to leaves caused by copper sprays.

The presence of injuries in the leaves has two effects: first, it causes an increased excretion of copper-dissolving substances (Arman & Wain, 1958) and, secondly, it increases the rate at which soluble copper is absorbed. A combination of these two factors may bring about localized spray damage. However, the increase in the absorption of soluble copper caused by the presence of extensive injuries in the leaves was sufficiently small for the conclusion to be drawn that injuries do not normally constitute a major route for the entry of copper into the leaf and it is probable that spray damage can occur in the absence of injuries.

Introduction

As was discussed in an earlier paper (Arman & Wain, 1958) excretions from leaves may bring about the solution of considerable amounts of copper from spray deposits of copper fungicides. If the leaves are able to absorb some of this dissolved copper, it could then be responsible for spray damage symptoms such as leaf spotting and browning and premature defoliation. It is of interest, therefore, to examine the manner in which copper is absorbed and the factors affecting absorption, since this may lead to a better understanding of the causes of copper spray damage.

The first attempt to detect copper in leaves which had been sprayed with Bordeaux mixture was made by Barker & Gimingham (1914). They removed the spray deposit by washing with acid and found copper to be present both in those leaves which had been damaged before they were sprayed, and in undamaged

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sprayed autumn leaves. It has been observed that when plants have been sprayed with Bordeaux mixture, the young unsprayed leaves are sometimes a darker green than normal; this may be evidence for the absorption and translocation of copper. Somers & Richmond (1957), using radioactive copper, have demonstrated the absorption by leaves of small amounts of copper from Burgundy mixture.

A study of the absorption of soluble copper by leaves of Canadian pondweed (*Elodea canadensis*), using radioactive copper, was made by Mazia & Mullins (1941). They used very dilute solutions of cupric chloride (as low as 10^{-10} moles per litre) and found that the copper was taken up from solution very rapidly, the leaf concentration rising to as many as $2{\cdot}9 \times 10^8$ times the concentration in the solution: some of the absorbed copper was subsequently eliminated from the leaves. Leaves thus have a very high affinity for copper and can absorb considerable amounts, even from very dilute solutions.

It has sometimes been noticed, and was found during the present investigations, that damage to plants by copper sprays is more severe in autumn; for example, Barker & Gimingham (1914) observed that in younger leaves the damage consisted of green or brown spots, whereas in older leaves there was general scorching and premature leaf fall. This greater susceptibility of autumn leaves may be due to the presence of a greater number of injuries. However, since the damage was not localized, Barker & Gimingham considered that autumn leaves might be more permeable to copper. In order to determine whether this is the case, experiments have been carried out to measure the capacity of apple leaves to absorb soluble copper at different stages of growth.

The interaction between an injured leaf and an insoluble copper fungicide is twofold; first, a greater amount of copper-dissolving substances is exuded than from an intact leaf (Arman & Wain, 1958) and, secondly, the injuries may provide a route for the copper to enter the leaf. A combination of these two factors could cause damage localized at the points of injury. The effect of injuries on the capacity of leaves to absorb soluble copper was therefore investigated.

Experimental

Methods used for measuring copper absorption by leaves

Sampling. Apple leaves free from visible injury were selected and detached from the tree. They were collected between 9.30 and 9.45 a.m. in good weather. The third and fifth leaves from the tips of vegetative shoots were picked; at the beginning of the summer the leaves were used immature, whereas later in the season they were fully expanded.

After picking, groups of third and of fifth leaves of similar size were selected and pairs of leaves, each pair containing one third and one fifth leaf, were made up. By this method of sampling pairs of leaves with total areas differing by not more than 1 cm.^{2} could be selected; in each experiment the area of one pair of leaves was measured and the figures for absorption were corrected to a total area of 40 cm.^{2} in order to standardize the results for different dates and varieties.

Assessment of copper absorption. The petioles of the leaves were sealed with wax and the leaves then immersed, with the petioles out of the liquid, in 75 ml. of solution for 4 hr., after which they were removed.

There are two ways in which the amount of copper absorbed may be assessed: (*a*) by analysing control and treated pairs of leaves and calculating the difference in copper contents, (*b*) by analysing the aliquots of solution before and after immersion of the leaves and calculating the amount of copper removed per unit of leaf area. The first method will include not only the errors inherent in analysis but also the sampling errors as between pairs of leaves. It was found that the first method gave lower figures than the second, a possible explanation for this is that rinsing the leaves before the analysis removed some copper loosely held on the leaf surface. Method (*b*) was used in the subsequent work.

Soluble copper was estimated by a modification of the method of Eden & Green (1940), using sodium diethyldithiocarbamate as the reagent. The method is as described earlier (Arman & Wain, 1958) with the difference that 5 ml. of amyl alcohol were used for the extraction instead of 2 ml. so that a range of 0–40 μg. of copper could be estimated.

Variations in the amount of copper absorbed of the order of 10–12% were found to occur between pairs of leaves of similar area immersed in the same solution.

The technique used and the preliminary experiments carried out are described in more detail by Arman (1957).

The variation in copper absorption with stage of leaf growth

Determination of the amount of copper absorbed by leaves of several varieties of apple from solutions containing 5, 10 and 20 p.p.m. of copper (as copper sulphate) were made on different dates throughout the summer of 1955. The results were all corrected to a leaf area of 40 cm.^{2} The varieties of apple used were: Cox's Orange Pippin, Worcester Pearmain, Newton Wonder, Edward VII, Grenadier and Monarch. The determinations were made on the following dates: 11–13 May, 14–16 June, 11–13 July, 10–12 August, 14–16 September and 20–22 October.

Leaves from the same tree of each variety were used for each concentration and all the trees used were in the same part of the orchard.

The results are plotted in Fig. 1.

Copper absorption in relation to leaf injury

Absorption by apple leaves. Measurements of absorption were made on comparable pairs of apple leaves, one set being uninjured and the other set carrying extensive freshly inflicted injuries (fifty pin pricks per leaf).

In the first experiment, measurements of copper absorption were made on pairs of leaves of two varieties from solutions of four concentrations. The results are given in Table 1.

A second experiment was carried out in which replicate measurements were made using Worcester leaves immersed in a 10 p.p.m. solution. The results are shown in Table 2.

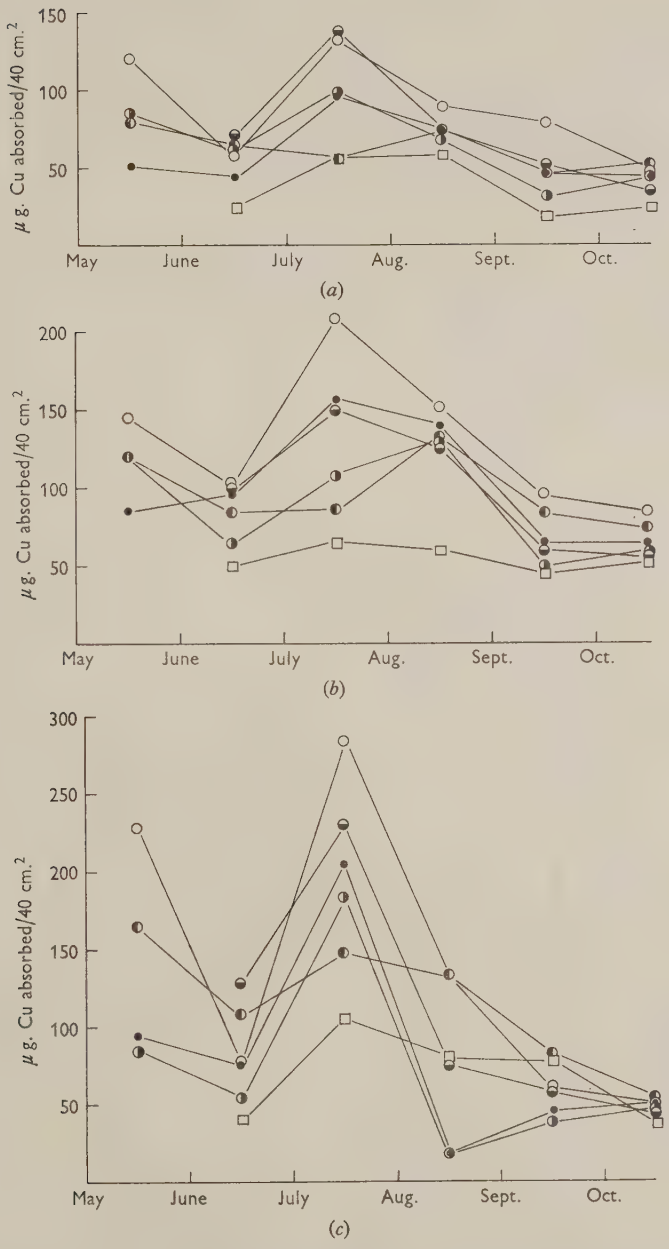

Fig. 1. Variation in copper absorption with stage of growth of apple leaves. (Treatment solutions: (*a*) 5 p.p.m., (*b*) 10 p.p.m. and (*c*) 20 p.p.m. copper as copper sulphate.) ●, Newton; ○, Cox; ◐, Worcester; ◑, Monarch; ◒, Grenadier; □, Edward VII.

Absorption by dwarf bean leaves. It was thought that apple leaves, although carefully selected, may have carried small injuries due to wind or insect damage. Other experiments were therefore carried out using pairs of dwarf bean leaves (*Phaseolus vulgaris*) from plants grown in a greenhouse. Three sets of leaves were used: (*a*) uninjured, (*b*) injured immediately before immersion in the solution and (*c*) injured while still on the plant 24 hr. before immersion.

The results are given in Table 3.

TABLE 1. *Copper absorption by apple leaves (var. Worcester and Cox)*

Cu concentration in p.p.m.	Absorption in μg./40 cm.²	
	Uninjured	Injured
Worcester leaves		
2	27	75
5	36	94
10	49	99
20	58	104
Cox leaves		
2	24	33
5	48	59
10	69	90
20	95	152

TABLE 2. *Copper absorption in* μg./40 cm.² *by Worcester leaves*

	Series A		Series B	
	Uninjured	Injured	Uninjured	Injured
	126	155	112	130
	119	133	104	144
	101	126	112	156
	115	155	120	122
	130	133	108	137
	115	126	126	148
	—	—	98	152
	—	—	112	126
Means	111·7	138·0	111·7	139·4

TABLE 3. *Copper absorption by bean leaves in* μg./40 cm.²

	Series A			Series B		
	Uninjured	Injured	Injured 24 hr. before	Uninjured	Injured	Injured 24 hr. before
	45	133	85	37	124	91
	53	165	101	48	120	97
	55	151	116	48	264	141
	49	126	91	48	172	135
	57	170	78	54	232	183
	50	144	105	54	225	136
Means	51	148	96	48	190	130

Discussion

It is apparent from the results described in the first part of this paper that the ability of apple leaves to absorb copper varies markedly with the stage of leaf growth and with the time of year (Fig. 1). Young leaves were found to take up a large amount of copper. Their capacity to absorb copper decreased in June and then rose to a maximum in July or August, followed by a decrease until leaf fall in late October or early November. These trends occurred consistently with six varieties and three concentrations of copper sulphate.

In some cases the leaves absorbed less copper from a 20 p.p.m. solution than from one containing only 10 p.p.m. This might be due to a toxic effect, assuming that leaves killed by copper absorb less than living leaves. Other experiments, to be described in a later publication, show, however, that dead leaves may take up more copper than living ones and also give some indication that in 4 hr. apple leaves may not be killed by concentrations below 100 p.p.m. (see Llewelyn, 1957). It would seem, therefore, that these differences in absorption must have some explanation other than toxicity.

The relatively high capacity of very young leaves to absorb copper may be related to the observations of McDaniels & Cowart (1944) that in apple leaves the stomata develop early and occupy a much larger proportion of the total area than in older leaves; there are also more hairs present on the epidermis. It is known that nutrients can be absorbed more rapidly through stomata than through the cuticle (Boynton, 1954).

The very high absorbing capacity of apple leaves in July and August and the subsequent decrease, however, cannot readily be accounted for on the basis of leaf structure. Since it is probable that leaf absorption depends on metabolism, as does that of root and tuber tissue, these differences in amount of copper absorbed may be related to changes in leaf metabolism.

It was suggested by Barker & Gimingham (1914) that apple leaves may be more permeable to copper in autumn. The present finding, however, that autumn leaves actually take up less copper than younger leaves shows that changes in susceptibility to copper sprays cannot be due entirely to changes in the permeability of the leaf to soluble copper. They must be at least partly due to some other factor, such as variations in the amount of copper dissolved by leaf exudations or in the susceptibility of the tissues to copper poisoning.

It is probable that older leaves carry a greater number of injuries, caused by wind, insect or fungal damage, than young leaves. The fact that such leaves absorb less copper suggests that in the field injuries are not an important route for copper entry. This is supported by the results described in the second part of this paper.

In the case of apple leaves the injuries inflicted (fifty pin pricks per leaf) caused an increase of 20–100% (with two exceptions) in the level of copper absorption; with dwarf bean leaves an increase of 200–300% occurred. According to these figures a single pin prick, which is quite a severe injury, would cause an increase in absorption of up to 2% in apple leaves and up to 6% in dwarf bean leaves. This

relatively small increase suggests that absorption by the control leaves is not due to undetected injuries. The presence of injuries which have been allowed to dry up causes an even smaller increase in absorption; furthermore, the amount of exudate which escapes from them is reduced (Arman & Wain, 1958). It would seem, therefore, that only those injuries inflicted near the time of spraying have a significant effect on the amount of spray damage.

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THE INFLUENCE OF CARBON DIOXIDE ON THE TOXICITY OF UN-IONIZED AMMONIA TO RAINBOW TROUT (*SALMO GAIRDNERII* RICHARDSON)

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(With 2 Text-figures)

The toxicity of solutions of ammonia to fish in different dilution waters showed a variation which was not entirely related to the concentration of the un-ionized ammonia molecule. Evidence is presented which shows that this variation can be attributed to the increase in the concentration of free carbon dioxide at the gill surface, which causes a decrease in both the pH value and the concentration of un-ionized ammonia present; the extent of the decrease depends on the initial concentration of free carbon dioxide in the bulk of the solution. Quantitative measurements of the relation between the free carbon dioxide concentration of the water and the toxicity of un-ionized ammonia are correlated with a theoretical estimation of the pH value of the water at the gill surface.

Introduction

It has long been known that the toxicity of a solution of ammonia or of an ammonium salt is increased by a rise in pH value, and several authors have shown that the toxicity of such solutions to fish depends upon the concentration of the un-ionized ammonia molecule present (Wuhrmann, Zehender & Woker, 1947; Wuhrmann & Woker, 1948; Downing & Merkens, 1955). However, subsequent investigations have suggested that the toxicity of solutions containing un-ionized ammonia to one species of fish, the rainbow trout, varies with the nature of the dilution water; thus attempts to correlate laboratory experiments with data obtained in a polluted stream showed an appreciable difference between the toxicity of un-ionized ammonia in the two investigations (Allan, Herbert & Alabaster, 1958), and differences were also found with waters of different chemical nature in carefully controlled laboratory experiments (Department of Scientific and Industrial Research, 1959). From a consideration of these data it was suspected that the essential differences between the various dilution waters might be their carbonate alkalinities and contents of free carbon dioxide.

Presumably it is the concentration of un-ionized ammonia at the gill surface which determines the toxicity of an ammonia solution. If it can be assumed that the carbon dioxide content of the respiratory water is increased by a constant amount as it passes over the gill surface, the pH value of the water at that surface will be depressed more when the carbon dioxide content of the original test solution is low than when it is relatively high, and consequently the concentration of un-ionized ammonia will be lowered at the gill surface to a greater degree in test

solutions containing a relatively low concentration of free carbon dioxide. If this hypothesis is correct, the toxicity of un-ionized ammonia would appear to decrease with a reduction in the concentration of free carbon dioxide in the test solution.

Methods

Tests were made in aquaria each containing 40 l. of solution, aerated with controlled mixtures of air and carbon dioxide at a rate of 4 l./min. Variations in the pH values of these solutions were within $\pm 0{\cdot}02$ units of the mean value. The temperature of the solutions varied with the air temperature from 18·7 to 20·7° C. during the 3 weeks in which the experiments were made, but were more constant than this for any one test. Solutions of ammonium chloride were prepared in untreated borehole water having a bicarbonate alkalinity of 250 p.p.m. as $CaCO_3$ and a total dissolved solids content of 420 p.p.m. Concentrations of free carbon dioxide in the water were calculated from the nomographs of Dye (1952); calculations of the un-ionized ammonia concentration in solution were based on the equation given by Albert (1951), using the pKa values quoted by Robinson & Stokes (1955). To bring the fish to a standard metabolic state at the start of each test, the rainbow trout (average length 5·6 cm.) were fed with a mixture of minced bullock's heart and liver and were then acclimatized for 18 hr. to the temperature and free carbon dioxide concentration of the test; they were then exposed in batches of ten to the solutions of ammonium chloride for a maximum period of 24 hr. The period of survival of each fish was taken from the time of immersion to the time when all respiratory movements had ceased. Where all ten fish died during the test, the distribution of survival times was log normal, but where only a few died, it usually appeared that there was a biological truncation of the distribution of survival times, that is, some of the more resistant fish survived for much longer than would have been expected from the log-normal distribution curve and the periods of survival of the more susceptible individuals. Median periods of survival were estimated by calculating the geometric means of the individual survival times when all ten fish died, but when only five to nine fish died during the period of the test, the median period of survival was estimated graphically (Bliss, 1937).

Results

From the results presented in Fig. 1, it is evident that the concentration of carbon dioxide in the test solution has influenced the toxicity of the un-ionized ammonia.

Discussion

If the amount of free carbon dioxide excreted by the gill of the fish were known, its effect on the pH value of the water, and therefore on the toxicity of un-ionized ammonia, could be calculated. Direct measurement of either the concentration of carbon dioxide or the pH value in the region of the gill surface would be difficult, but estimates of these values can be obtained indirectly. The increase in the con-

centration of carbon dioxide (as mg./l. CO_2) in the water leaving the gills is given by the relation

$$C \times \text{R.Q.} \times \frac{\text{mol wt. } CO_2}{\text{mol wt. } O_2} \times \frac{P}{100},$$

where C is the concentration of dissolved oxygen in the test solution (in mg./l.), and P is the percentage removal of dissolved oxygen from the water by the fish.

According to Van Dam (1938), salmonid fishes absorb about 80% of the oxygen from the water flowing over the gills, and we have assumed that the respiratory quotient of trout fed on a predominantly protein diet would be 0·8. Using these values, the concentration of carbon dioxide originally in the water would be increased during its passage over the gill epithelium by an amount equal to 0·88 of the concentration of dissolved oxygen in the water.

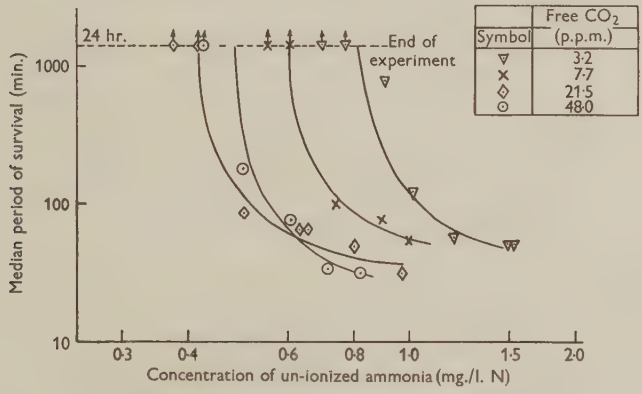

Fig. 1. Toxicity of un-ionized ammonia to rainbow trout in water containing different concentrations of carbon dioxide

As the carbon dioxide is presumably excreted along the length of the secondary lamellae of the gills, a pH gradient will be formed, and the average pH value of the water at the gill surface has been taken as that which would be produced when half the carbon dioxide has been excreted. It is now possible to calculate the average pH value of the water at the gill surface from the concentrations of dissolved oxygen and free carbon dioxide, bicarbonate alkalinity, and temperature of the water, and from this the average concentration of un-ionized ammonia at the gill surface can be derived for any given concentration of total ammonia in the bulk of the solution.

For any given conditions of temperature and dissolved oxygen, and within any one population of fish, the average concentration of un-ionized ammonia required at the gill surface to kill 50% of the population in a given time should be a constant. The concentrations of un-ionized ammonia in the bulk of the solution which killed 50% of the test fish after 500 min. (500 min. TL_m) at each of the four concentrations

of free carbon dioxide have been calculated by probit analysis and are given in Table 1. Also in the Table are the corresponding concentrations of un-ionized ammonia, calculated by the method described above, which would have been present at the gill surface. These latter values are much more nearly constant than the concentrations for the test solutions, which strongly suggests that the assumptions which have been made are valid.

Table 1. *Estimated 500 min. TL_m concentrations of un-ionized ammonia in the test solutions and the corresponding average concentration at the gill surface*

Conditions in the bulk of the test solutions			
pH value	Free CO_2 (p.p.m.)	500 min. TL_m concentration of un-ionized ammonia (p.p.m. as N)	Average concentration of un-ionized ammonia at gill surface (p.p.m. as N)
8·2	3·2	0·84 (0·79–0·90)	0·39 (0·37–0·42)
7·8	7·7	0·62 (0·56–0·68)	0·42 (0·38–0·46)
7·37	21·5	0·42 (0·38–0·46)	0·36 (0·33–0·39)
7·0	48·0	0·49 (0·46–0·53)	0·45 (0·42–0·49)

Figures in brackets are 95 % fiducial limits.

Although the pH value corresponding to a given concentration of free carbon dioxide depends upon the alkalinity of the water, it can be shown by calculation that the decrease in pH value for an increase in free carbon dioxide from one concentration to another is constant, and independent of the alkalinity. Thus, at a temperature of 18·5° C., with a carbonate alkalinity of 25 p.p.m. as $CaCO_3$, an increase in free carbon dioxide from 5 to 10 p.p.m. reduces the pH value from 7·03 to 6·72, while with an alkalinity of 250 p.p.m. at the same carbon dioxide concentrations the pH values would be 8·00 and 7·69, the fall in pH value being the same at both alkalinities. Furthermore, over the range of pH values of interest here, the concentration of un-ionized ammonia increases with almost constant proportionality for equal increments in pH value. From this it is clear that the difference in pH value between the bulk of the test solution and that at the gills of the fish will depend mainly on the free carbon dioxide in the bulk of the test solution, and if it is assumed that the concentration of un-ionized ammonia required at the gill surface to produce a 500 min. TL_m among the fish is 0·4 p.p.m. as N (the overall average value from Table 1), it is possible to relate the concentration of free carbon dioxide in the test solution to the concentration of un-ionized ammonia in the test solution which is required to produce this toxic effect. This has been calculated, and the results are shown as the continuous line in Fig. 2. The circles are from the data of the present experiment, and the crosses are values from earlier experiments made in waters of low carbonate alkalinity (Downing & Merkens,

1955; Department of Scientific and Industrial Research, 1959). The agreement of these earlier data with the curve provides further evidence in support of the hypothesis.

It appears from this evidence that the method used to estimate the pH value at the surface of the rainbow trout gill is valid, so a similar approach could presumably be used to assess the toxicity of all poisons which are affected by pH changes within the range tolerated by fish.

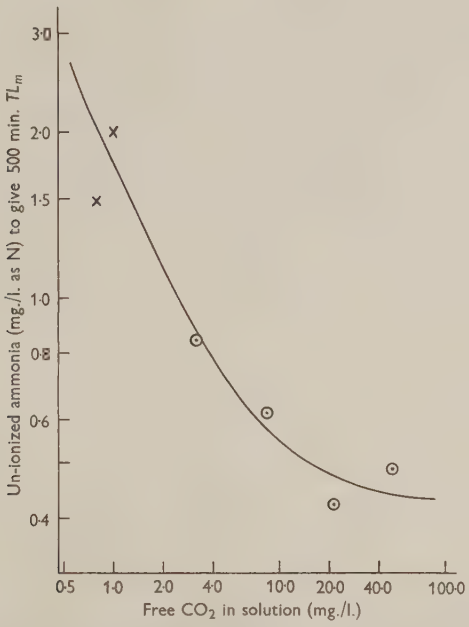

Fig. 2. Relation between free carbon dioxide and the concentrations of un-ionized ammonia producing a 500 min. median period of survival of rainbow trout at 20° C. The circles show results of the present experiment, the crosses those of earlier work. The line is the theoretical curve, assuming the average concentration of un-ionized ammonia at the gill surface to be 0·40 mg./l. as N.

Mr A. C. Wakeford assisted with the experimental work. This paper is published by permission of the Department of Scientific and Industrial Research.

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(*Received* 13 *November* 1959)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Meetings of the Association were held in London on 23 October and 4 December 1959 and on 8–9 January, 12 February and 11 March 1960. The full list of speakers and papers at the 1959 Meetings is given in the Report of the Council (p. 442).

Abridged versions of the following ten papers are presented below:

Meeting of 23 October

Symposium on the control of harmful birds

Economic ornithology. By Mr Harry V. Thompson.

The sense of taste in birds. By Dr C. J. Duncan.

The use of narcotics in catching harmful birds. By Mr R. J. P. Thearle.

The biology and economic importance of the bullfinch. By Mr E. N. Wright and Mr D. D. B. Summers.

Population dynamics of the wood-pigeon (*Columba p. palumbus*) and methods of control. By Mr R. K. Murton.

Meeting of 4 December

The bioassay or residues of insecticides in soil. By Mr G. A. Wheatley and Mr J. A. Hardman.

A transmissible disease of cultivated mushrooms ('watery stipe'). By Miss Doreen Gandy.

Cecidomyiidae as pests of cultivated mushrooms. By Mr I. J. Wyatt.

Meeting of 12 February

Growth of the potato plant. By Messrs N. W. Borah, R. L. Burt and D. W. R. Headford, Professor F. L. Milthorpe and Miss E. M. Sadler.

The bourse shoot as a factor in the growth of apple fruits. By Mr D. L. Abbott.

Ann. appl. Biol. **48** (2), 405–408.

ECONOMIC ORNITHOLOGY

By Harry V. Thompson

Ministry of Agriculture, Fisheries and Food, Tangley Place, Worplesdon, Surrey

It is always as well to begin by defining one's terms, and to the natural man economic ornithology undoubtedly meant a bird for the pot—a theme that could appropriately be developed in this meeting place, for the present director of the Zoological Society dedicated his book (Matthews, 1951) *Wandering Albatross*, 'to bird lovers especially those who love them piping hot, well browned and with plenty of bread sauce'.

But we have met to discuss the control of *harmful* birds and although I shall refer later

to gamekeepers and their killing of raptors I will, reluctantly, save my powder as far as game birds and wild-fowl are concerned.

In relation to agriculture, birds of economic importance are those which directly depend on the activities of man for much of their food. The significance of the damage done by these birds has grown with the development of our system of intensive agriculture and interspersed woodlands, which offers ideal conditions of food and shelter for the wood-pigeon, rook, starling and house-sparrow and in some areas the bullfinch.

While the scaring and killing of harmful birds has been practised for centuries, the last two decades has seen a more scientific approach to the problem in this country. Stimulated by the need to conserve food during the last war, the Agricultural Research Council sponsored work at Oxford on the wood-pigeon (Colquhoun, 1951) and the rook (*A.R.C.*, 1948). The publication by Hartley, in 1948, of *The assessment of the food of birds* came as a timely reminder that much classification of wild birds as 'beneficial' or 'harmful' is extremely naïve. It is a common practice to attempt to balance the apparent good a bird does, by eating weed seeds and agriculturally undesirable insects, against the damage it does to crops. But as Hartley points out it has seldom been proved that birds exercise any control upon the numbers of insect prey. This same point was taken up by Evans at a meeting of this association in March 1951, in reference to the negligible importance of rooks in controlling wireworms. He continued by emphasizing the need for scientific investigation of problems of bird damage and secured the appointment of the Ministry's first official ornithologist in the following year. Concurrently with this more realistic approach to harmful birds there has been increased public concern over bird protection. This culminated in the Protection of Birds Act (1954) which drew together legislation from 1772 to 1952, and, in effect, gives most birds protection, at least for part of the year, even if they do cause damage. However, under §4(2)(*a*) of the Act a person shall not be guilty of an offence against the Act 'by reason of the killing or injuring of, or an attempt to kill, a wild bird other than a bird included in the First Schedule to this Act if he satisfies the court before whom he is charged that his action was necessary for the purpose of preventing serious damage to crops, vegetables, fruit, growing timber or any other form of property or to fisheries'.

A small number of birds, only some twenty species, on the 2nd Schedule of the Act may be killed or taken at any time by authorized persons and it is, of course, with these 2nd Schedule birds that our research is mainly concerned.

A similar situation exists in the United States of America. Bird control in California, for instance, is almost as old as agriculture in the State and those species which were the main offenders over 70 years ago—especially the linnet, *Carpodacus mexicanus*, and the horned lark, *Otocoris alpestris*—are still abundant. Depredations on rice by red-winged blackbirds, *Agelaius phoeniceus* and other species are common wherever rice is grown and measures of control are a perennial necessity (Neff & Meanley, 1957). Nevertheless, official United States agencies engaged in this work are adjured to remember that birds are very generally protected by State or Federal laws, and that the conservation of migratory, insectivorous, game and song-birds is a major consideration.

Assessment of a problem of damage

This is seldom a simple matter. Even in the relatively straightforward case of the killing of lambs by ravens (mentioned in a general paper by Ridpath & Murton, 1956) many hours of observation were necessary to establish the fact of damage in one small area. Quantitative assessment of such damage, or of similar attacks on lambs by carrion or hooded crows is much more difficult.

Our work is greatly stimulated by suggestions from advisory officers and inquiries from farming and other organizations, but they are naturally mainly concerned with methods of reducing damage rather than measuring it. While research on control techniques must proceed concurrently, it is, nevertheless, essential that the approach to any damage problem should be an ecological one. The nature and extent of the alleged damage must be investigated to find if it is local or widespread, seasonal or continual, occasional or perennial. The

abundance and natural fluctuations of the birds causing the damage must be studied and their movements, feeding habits, behaviour, breeding biology and predators observed. This invariably calls for field observations, marking studies, some method of surveying numbers and sometimes crop or gizzard analyses.

Experience in this country and elsewhere has shown that fundamental work on the ecology of a pest, combined with studies of methods of control, is ultimately far more fruitful in reducing damage than the purely *ad hoc* approach.

Methods of control

(*a*) *Changes in husbandry*. Manipulation of the habitat is sometimes a much more practicable method of control than direct action against a harmful species. This is illustrated by four examples from different parts of the world.

The first case does not concern agriculture but is similar to the problem of gulls on airfields in Britain with which we have been concerned. At the United States naval airbase on Midway atoll at the western end of the Hawaiian chain of islands, resident sea birds, mainly the Laysan albatross, *Diomedea immutabilis*, constitute a hazard to aircraft. Kenyon, Rice, Robbins & Aldrich (1958) showed that a policy of destruction would involve killing tens of thousands of albatrosses annually and would have to be continued indefinitely. They suggested as a practicable alternative, the clearing of bushes and trees for several hundred yards on either side of the runways and the flattening of the earth. These measures were designed to eliminate updraughts of air and consequent soaring of the albatrosses in the vicinity of the air strips.

In the Northern Territory of Australia the magpie goose (*Anseranas semipalmata*) was said to be destroying experimental rice crops and threatening the whole future of the commercial rice-growing project. Mass destruction of the birds was contemplated, but investigations by Frith (1960) showed that much of the damage attributed to the geese was, in fact, caused by lack of water control and inappropriate agricultural methods. Some goose damage did occur, particularly to ripening grain in the vicinity of nesting colonies. Since magpie geese nest in water 1–3 ft. deep, Frith proposed that this particular problem should be dealt with by raising or lowering the water level in these areas. This would force the geese to breed, and feed, at a greater distance from the rice fields. Other aspects of the goose–rice complex require study but this ecological approach is most promising.

Bullfinch damage to fruit buds, as Wright will illustrate this afternoon, is most severe near hedges or woodlands and the damage can be reduced by removing any unnecessary natural cover on fruit farms.

The final instance is the predation of lambs by eagles on the Isle of Lewis, reported by Lockie & Stephen (1959). They found that eagles increased in numbers on Lewis about 1946, at the same time as mountain hares, grouse and rabbits began to decline. It was suggested that the stocks of sheep were increased beyond the capacity of the habitat to support them, and that there was in consequence more lamb and sheep carrion available for the eagles. The observers concluded that the problem of eagles taking live lambs will not be permanently solved by shooting, only by improved land use.

(*b*) *Use of repellents*. This is a diffuse subject on which there is little published information, and some of that is conflicting in that repellents used successfully at one place and time may not be so successful under different conditions. Scaring devices have been used for many centuries but have seldom been critically evaluated. A repellent may exercise its effect through one or more of the visual, olfactory, auditory, tactile or gustatory senses; a review of current work by Duncan, Wright & Ridpath is in the press and some details will be given later today. Duncan's work on taste was, indeed, undertaken to establish a rational basis for the use of chemical repellents.

Damage to some crops takes place only during short periods and it is generally agreed that if it could be prevented by the use of deterrents this would be much more satisfactory than killing the birds. But it must be admitted that up to the present repellents have met with only limited success.

(*c*) *Destruction.* Inevitably, a good deal of our work is concerned with direct action against harmful birds. From 1953–9 organized wood-pigeon shoots have killed an estimated 6½ million birds and been supplied with some 16 million subsidized cartridges; independent shooters using decoys have killed about 4¾ million birds with some 10 million cartridges. A study of the biology of the wood-pigeon, as you will hear this afternoon, has led to the official adoption, this year, of nest destruction as an ancillary method of control.

Field studies by Wright and Summers have led to greatly improved techniques for trapping bullfinches, and it is hoped that work now being planned will result in the more effective use of traps for corvids.

The use of narcotics in controlling bird pests has been attended by some publicity during the last few years and unpublished work by Ridpath, Thearle, McCowan and Jones will be reported today.

In conclusion, I may say that the aims of agriculture, public amenity, game preservation and other aspects of wild-life conservation can never be identical although they have many interests in common. There must be a compromise which fits wild life into a balanced pattern of land management, of which farm pest control is an integral part. There is much need for education here, of the keeper who kills protected hawks without realizing that they may have a negligible or even beneficial effect on his game stocks, as well as of the landholder who imagines that a species of bird so biologically successful as to be classed as a pest can be completely annihilated.

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Ann. appl. Biol. **48** (2), 409–414.

THE SENSE OF TASTE IN BIRDS

By C. J. Duncan
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(With 2 Text-figures)

Introduction

An understanding of the physiological mechanisms underlying the sense of taste in birds and the role they play in avian behaviour would be extremely useful in the investigations on possible chemical repellents. Such repellents could be sprayed on crops and so reduce damage by birds. Two centres of research on the sense of taste in birds have developed, one in Germany and the other in the U.S.A. and in general their conclusions appear to disagree. Engelmann (1940) concluded that hens base their preference for their food primarily on the shape of the grain and only secondarily on the colour, the taste having little effect on their choice. However, a series of preference tests, again using hens, has been described by Kare, Black & Allison (1957) and they found convincing evidence of wide taste discrimination when various chemicals were added to the drinking water. These apparently conflicting results are discussed in greater detail below.

Problems in sensory physiology can generally be attacked in three ways and in the present investigation on the sense of taste each of these methods has been tried:

(1) Histological studies, with the aid of which the structure and position of the sensory receptors can be determined.

(2) Electrophysiological investigations which provide direct information of the intensity and nature of the stimuli to which the birds are sensitive.

(3) Preference tests and behaviour studies.

In most of the previous work hens have been used as the experimental material, but it would be unwise to generalize from the results obtained since the hen is a domestic species. In the present work the feral pigeon (*Columba livia* var. Gmelin) has been studied. It is easy to keep in captivity, it is sufficiently large for electrophysiological studies and the pigeons are of considerable commercial interest as pests. Much of the work is not described in detail because some of it will appear in full elsewhere and other parts are still in progress.

Histology

The sensory receptors of taste are termed taste buds and consist of ovoid clusters of cells lying in cavities in the stratified epithelium of the tongue. They resemble mammalian taste buds in appearance, having fusiform gustatory cells which are surrounded by a sheath of supporting cells. However, the most noticeable feature is the small number of taste buds found. Moore & Elliot (1946) report a maximum of fifty-nine in the pigeon (*C. domestica*) and these are confined to the base of the tongue, the anterior region being cornified.

Sections of the tongues of the feral pigeon showed the same arrangement with somewhat fewer buds. In the bullfinches examined, the totals found were seventy-seven buds in a juvenile bird and forty-two and fifty-one in two adults. Again, the buds were confined to the posterior end of the tongue and were frequently associated with the openings of the mucous glands. These figures are in contrast with the estimated numbers in mammals, there being a total of about 9000 buds in man and 17,000 in the rabbit; the results would suggest that the sense of taste in many birds is rudimentary or relatively unimportant.

Electrophysiological studies

When suitably stimulated by chemicals, the taste buds initiate nervous impulses which are carried to the brain by the IXth cranial nerve. The experimental technique involves

measuring the very small changes in potential which occur in the nerve when such nerve impulses are generated.

The pigeons were anaesthetized by an injection of urethane and were then dissected with the aid of a binocular microscope to locate the lingual branch of the IXth nerve. The nerve sheath was removed with fine needles and split longitudinally into small bundles, each containing a few fibres. The nerve was cut centrally to ensure that any nervous impulses recorded were, in fact, proceeding from the taste buds and not descending from the brain. One of the nerve bundles was placed on a silver/silver chloride electrode and this was moved by means of a micromanipulator. In this way the fibres were lifted clear of the surrounding tissue and, at the same time, any changes in potential due to nervous activity could be conducted from the electrode to an amplifier, the amplified signals being displayed on an oscilloscope. Conventional, single-sided, a.c.-coupled amplifiers were used and the smallest test signal which could be distinguished was about 7 μV. The initiation of gustatory impulses was then tested by the perfusion of the test solution through a cannula in the trachea and so over the tongue.

However, the results obtained were disappointing. Generally no nerve activity was recorded, although occasionally a burst of impulses was found after stimulation with 0·5 % acetic acid. These results were not repeatable at will, although some of the other, non-gustatory fibres were certainly functioning. Thus, if the lingual branch was not cut centrally, the activity of the motor fibres (probably supplying the mucous glands) was always detected. The unsatisfactory results are probably due to the faults and a lack of refinement in the technique rather than to an absence of gustatory impulses. These latter are carried in very small nerve fibres in mammals and sections of the lingual branch of the IXth nerve of the feral pigeon show that the smallest fibres are correspondingly small and a preparation containing a small number of such fibres deteriorates quickly. Since the number of taste buds is so relatively low, it is probable that only a very small proportion of the fibres in the lingual branch are concerned with the conduction of afferent, gustatory impulses, a factor which further increases the difficulties of obtaining a satisfactory preparation. The same apparatus and technique worked quite satisfactorily when tested with frogs or rats.

Preference tests

Kare and others (1957) have described an extensive series of preference tests with the fowl in which groups of twenty chicks were given a free choice of fluid in one of two drinking bowls. One of these 'waterers' contained the flavoured solution whereas the other contained water. A comparison of the two volumes drunk gave a measure of the preference or rejection by the birds for the particular flavour. Thus, the birds showed a strong preference for a two-parts per thousand solution of butter-type flavour, whilst they rejected solutions of oil of patchouli, oil of costus and honey at the same concentration. Difficulties were encountered because the birds (as with many other animals) developed a marked preference for a waterer in a particular position, irrespective of the solution which it contained, and also because the birds did the greater part of their drinking in the early hours of the morning.

TABLE 1. *Daily schedule for test birds*

Treatment	Time (hr.)	Duration (hr.)	Fluid supplied
Deprivation	17·00–09·30	16½	No fluid
Test	09.30–11.30	2	Test solution
Rest	11·30–13·00	1½	No fluid
Maintenance	13·00–17·00	4	Distilled water

In the present experiments with feral pigeons a modification of the 'one-bottle' technique, developed by Weiner & Stellar (1951) when studying rats, was used. The pigeons were kept in separate cages and subjected to a regular, artificial day and night. The room temperature was thermostatically regulated as far as possible, although the temperature rose slightly

above this level at noon on very hot days. The birds were divided into groups of eight at random and supplied with excess food. After a period of habituation, they were kept on a strict daily schedule, outlined in Table 1. Each bird was presented with only one drinking fountain, which contained the test solution *or* water, for 2 hr. each day and the volume consumed was measured. After this came a rest period (no fluid supplied) and then the afternoon maintenance period, when a single tube of water was supplied. During this latter time the birds were able to satisfy any fluid deficiency. Finally, no fluid was given during the 16½ hr. overnight period. It was therefore possible to obtain a direct comparison between the average volume per bird of test solution drunk and the corresponding average value when distilled water was supplied in its place on the following day during the 2 hr. test period.

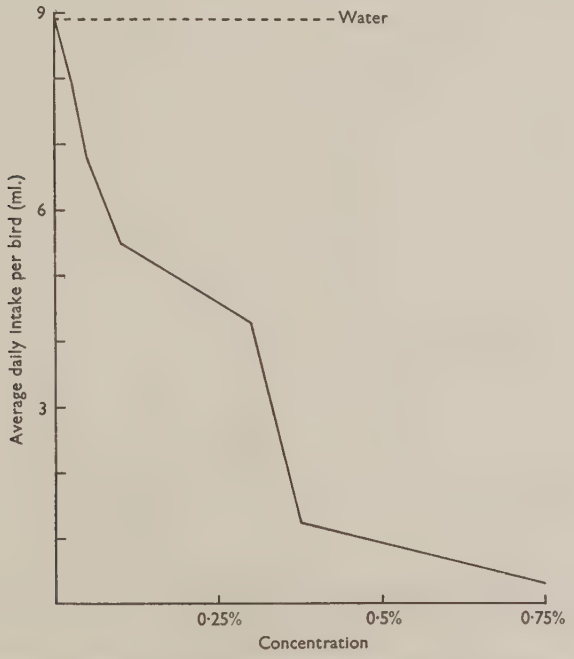

Fig. 1. Acceptance of acetic acid at different concentrations. The comparison with water (0 %) is emphasised. Ordinates = mean intake per bird of test solution in ml. during the daily test period.

Each of the eight birds in any one group was presented with the same concentration of test substance during the test period on 3 successive days, giving twenty-four readings. Every fourth day distilled water was given as the test fluid, before a different concentration of the substance being tested was investigated.

The substances tested were all simple and are readily classified within the four classical taste modalities, sweet (sugars), sour (acids), salt and bitter (e.g. quinine)—being chosen for this reason. The results are briefly considered under these headings:

(1) *Acids.* Both acetic and hydrochloric acids were taken in very much smaller quantity than distilled water, even at very low concentrations. With acetic acid (see Fig. 1) there was an increasing rejection with increasing concentration, with an average intake/bird/test of

0·4 c.c. at 0·75 %. Conspicuous rejection was also found in another group of birds tested with hydrochloric acid.

(2) *Sugars.* Glucose and sucrose were tested and in both cases the birds appeared to be almost indifferent, although there was a slight preference for sucrose at concentrations around 15 %, which proved to be statistically significant.

(3) *Quinine hydrochloride.* Again a marked rejection was found with a significant drop in consumption at a concentration as low as 0·1 %.

(4) *Chlorides.* Separate groups of pigeons were given sodium, calcium and potassium chlorides and in all three groups a similar pattern of preference was found. At the lower concentrations (see Fig. 2) the birds showed a slight preference, but with increasing concentration a progressive rejection was found.

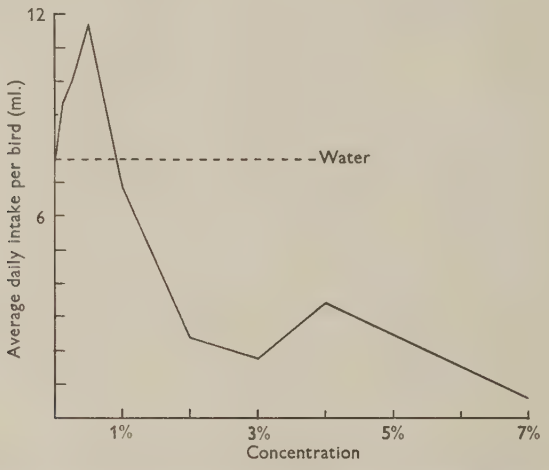

Fig. 2. Acceptance of sodium chloride at different concentrations. The comparison with water is emphasised. Ordinates = mean intake per bird of test solution in ml. during the daily test period.

Considerable variation between individual birds was observed, both in total daily fluid requirements and in taste preferences. The mean daily fluid intake varied from 14 c.c. (bird no. 44) to 62 c.c. (bird no. 57) and this variation was also reflected in the consumption of the test fluids, resulting in a large standard deviation at each concentration. Nevertheless, each bird retained, in general, its own pattern of drinking, not only over the 3 days at any one concentration, but throughout the experiment. A heavy drinker maintained a high level of fluid intake throughout. Bird no. 15 proved exceptional and his daily consumption during the 2 hr. test period remained almost constant, irrespective of the fluid supplied. For example, when offered 0·5 % hydrochloric acid, his mean daily consumption was 13·4 c.c., whereas the other seven birds in the group drank a corresponding average per bird of 0·3 c.c.

The birds used were all mature and had therefore previously experienced a variety of tastes. In the development of repellents, it is the responses of such wild birds which are of interest, there being differences in taste preferences between hand-reared, naïve birds and birds captured in the wild.

It was not possible to repeat the experiments, using birds in which the lingual nerve was cut, but it is hoped that this will be done in the future.

Repellency

E. N. Wright (unpublished), using a similar but modified technique, has extended the preference tests and investigated the effects of offering solutions of the ingredients of some commercial repellents as the test substances. The birds used were again feral pigeons. In the control experiment there was no significant difference between the intakes of a 1% suspension of kaolin (with a turbid appearance) and water. Significant reductions of intake, when compared with the water standard, were found with 1% solutions of tetramethyl thiuram disulphide, diphenyl guanidine and β-naphthol. The deterrent effect of a 1% solution of anthraquinone, although statistically significant, was less marked.

The primary test of a successful bird-repellent substance (see Duncan, Wright & Ridpath, 1960) is that when it is applied to the animal's food it will repel hungry birds. Simple tests with groups of eight feral pigeons kept together in the same cage, following a previous 15 hr. starvation, showed that a liberal application of 0·07N acetic acid, or 4% potassium alum to the food (maize) had little deterrent effect. The substances chosen had both shown a marked repellent effect when offered to feral pigeons in the preference tests. When the birds were offered the choice between treated and untreated grain, they generally ate slightly more of the food from the untreated sample, although they frequently continued to eat from the bowl at which they began.

Przygodda (1955) has performed similar experiments on house sparrows, using the commercial repellent Morkit and only at the relatively high concentration of 200 g. Morkit per 50 kg. food was there a significant choice in favour of the untreated grain. Following a series of tests in Germany, Engelmann (1940, 1943) concludes that hens base their preferences for certain cereals primarily on shape and secondarily on colour, whilst he found that taste had little effect. Experiments in which the grain was soaked in flavouring solutions similarly suggested that tactile stimulation will predominate over even a strong taste stimulation. Thus, compared with the results obtained by coating the grain with talc or waterglass and so altering their tactile sensation, the effect of added flavourings remained small. Duncan, Wright & Ridpath also tentatively conclude that sticky substances are repellent, although their use in practice is limited at present, due to phytotoxicity problems.

The experimental data suggest that, in spite of their apparently limited taste-receptive equipment, birds are able to detect the presence of a variety of gustatory stimuli, some of which are attractive and some repellent, when compared with water. The observed repellency usually increases at the higher concentrations of the test substance. The observations, however, do not enable us to decide whether birds can distinguish between, say, salt and sour taste stimuli; elaborate training experiments would be needed to determine this point. However, it does seem probable that taste is not an important factor in the selection of food, and that hungry birds will not be readily deterred from eating by the application of repellent substances, although many birds avoid insects which apparently have an unpleasant 'taste'. The true nature of this stimulus is not known.

This work was done during the tenure of a Civil Service Research Fellowship. It is a pleasure to be able to thank Prof. Sir Bryan Matthews, F.R.S. for his permission to work at the Physiological Laboratory, Cambridge, and for his advice and encouragement. I should also like to thank Mr P. E. K. Donaldson for much technical advice. I am grateful to all the members of the Land Pests Research Branch of the M.A.F.F. for their practical assistance in many ways and in particular I should like to thank Messrs R. J. P. Thearle and D. D. B. Summers for their help with the cage tests and Dr I. Thomas and Messrs H. V. Thompson, M. G. Ridpath and E. N. Wright for their suggestions and helpful criticism.

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THE USE OF NARCOTICS IN CATCHING HARMFUL BIRDS

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Damage by wood-pigeons, rooks, crows and jackdaws to crops, and by feral pigeons and house-sparrows to stored foods and urban buildings, is difficult to measure, but can be extensive. Present methods of control are costly and not particularly efficient, so new methods of control must be devised. Under the Protection of Birds Act, 1954, it became possible to use, under licence, poisoned or stupefying bait to kill or take certain wild birds, regarded as harmful. Narcotic baiting has the theoretical advantage over control by rapid-killing agents that if harmless or legally protected species are caught, they may be released on recovery.

Considerable work on the use of α-chloralose for catching birds has been done abroad, mainly in France. It was decided to concentrate on using chloralose, but other substances were tested in the laboratory. The work can be divided into three main sections—laboratory tests, rural trials and urban trials.

The laboratory work included the testing of seven candidate narcotics and poisons. Apart from chloralose, nicotine was the only substance that showed promise, but it was not possible to test it in the field.

An LD 50 determination for α-chloralose to feral pigeons was carried out and a figure of about 130 mg./kg. was obtained.

Four different methods of administering chloralose to feral pigeons were compared, as it had been observed that different methods produced different effects. The results showed that treated grain and flour pills were more effective than gelatine capsules, either with or without grain, and that flour pills were most satisfactory for laboratory trials.

The rural trials were directed against corvids and wood-pigeons. Those on corvids were to confirm that the success of the work carried out in France could be achieved in this country. In four trials fourteen birds were taken (six rooks, eight jackdaws) of which three died. All four trials used chloralose at 0·5 % by weight in bait. Against wood-pigeons preliminary trials took place in 1955 using chloralose at 2·5 % by weight in grain bait. The first trials lasted 10 days and the second 5 days: no birds were seen to feed. In 1956 more extensive trials were carried out using chloralose at 2·5 % by weight, but failed, due to the

reluctance of wood-pigeons to feed on the bait. In 57 baiting days only seven wood-pigeons (of which one died) and one stock-dove were taken.

The urban trials were directed mainly against feral pigeons. A series of fifty trials were carried out, comprising five sets of ten trials each, using concentrations of α-chloralose between 0·5 and 2·5 % by weight in bait. All trials were done at dawn. It was found that lower concentrations of chloralose resulted in lower mortality but not lower efficiency. A total of 1544 feral pigeons was taken. In addition, five operations attempting complete clearance of feral pigeons from infested sites were carried out. Efficiency of clearance varied between 50 and 80 %, and a further 911 pigeons were taken.

Only two trials have been carried out solely to catch house-sparrows; neither was successful. Sparrows have been caught incidentally, however, in the feral pigeon trials. In five trials eighty-eight house-sparrows were taken.

The use of α-chloralose, in urban areas, appears to be a reasonably effective method of controlling birds that will readily accept a grain bait. Provided adequate precautions are taken, the risk to protected birds and other animals is small. In rural areas risks are greater and in the case of the wood-pigeon it is still necessary to find an attractive bait.

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THE BIOLOGY AND ECONOMIC IMPORTANCE OF THE BULLFINCH

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The economic importance of bullfinches, like that of so many other birds, results from their feeding habits. During the winter their diet consists largely of tree buds, especially those of fruit trees, and in areas of extensive fruit cultivation the bullfinch can undoubtedly be a pest.

This is not a new problem. As long ago as 1618 Lawson wrote: 'The Bull-finch is a devourer of your fruit in the bud', and by an Act of Parliament in the reign of Elizabeth I the sum of one penny was offered for every bullfinch's head. The records of the churchwardens of Tenterden, in Kent, show that a number of such payments were made between 1628 and 1680, so evidently the bullfinch was considered an important pest even then. In 1726 R. Bradley, Professor of Botany in the University of Cambridge, published a *Treatise of husbandry and gardening*, which contains a letter about the destructive habits of bullfinches but asserting that '. . . it is a little worm that they seek . . .'. This view is still popular today although there is no evidence to support it. Some years ago Dr A. M. Massee of East Malling Research Station examined 2000 Conference pear buds, taken from trees that were being attacked by bullfinches, but found no evidence of insect infestation. Analyses of the stomach contents of bullfinches shot on fruit farms show quite clearly that it is the bud tissues that are eaten; insect material is rarely present. Robert Mudie, writing in 1834, expressed the view that bullfinches attack trees '. . . that have their buds in an advanced state, and with a considerable amount of farinaceous matter accumulated in them'. This hardly holds true today, as damage may begin in November and continue until blossom time. In 1935 the British Trust for Ornithology began an inquiry into the bud-eating habits of birds and the findings were published by Fryer (1939). The author concluded that the species mainly concerned were the bullfinch and the house-sparrow but the part played by other birds required further investigation.

About 1950 the National Agricultural Advisory Service began to receive many complaints from fruit growers regarding bird damage to dormant buds and English (1953) and Laflin (1953) carried out numerous experiments in an effort to overcome this problem. None of the methods investigated gave any significant protection from bird attack and in 1953 it was agreed that research should be undertaken by Infestation Control Division of the Ministry

of Agriculture. In view of the generally unsatisfactory results obtained by the empirical approach it was decided to investigate the biology of the bullfinch in the hope of obtaining a better understanding of the principles underlying the problem.

The dormant buds of fruit trees are, broadly speaking, of two types—fruit buds (i.e. those destined to produce blossom) and leaf buds (i.e. those producing extension growth and leaves). The loss of fruit buds results in fewer blossoms and, potentially at least, less crop. In many varieties the damaged buds are replaced the following year through the development of ancillary buds. In a few varieties, however, notably some gooseberries and plums, no regeneration of fruiting points occurs and damage is therefore cumulative. The crop from severely damaged trees, if they produce any crop at all, is usually of a high quality but this never compensates financially for the loss in quantity. Damage to leaf buds rarely reduces the number of leaves to the point where the physiology of the tree is noticeably affected. However, owing to the loss of growth buds, it may be almost impossible to train young fruit trees to a good shape—a factor that affects the cropping potential throughout the life of the trees.

Bullfinches strip buds very quickly and systematically. Alighting near the tip of a branch they work towards the centre of the tree taking every bud; when they reach the older wood, which bears fewer buds, they fly out to the tip of another branch and repeat the process. Because the birds do not necessarily alight right on the end of the branches, it is common for the terminal buds to be left undamaged. A bullfinch takes buds at an average rate of about thirty per minute and this is roughly equivalent to one bird stripping two gooseberry bushes in an hour. As they usually feed in parties, of from five to ten birds, this high rate of feeding seems sufficient to explain the sudden appearance of extensive damage.

The measurement of bullfinch damage might at first appear to be a relatively straightforward, if somewhat arduous, task. By counting the number of buds that have been pecked out, and the number remaining, a figure for damage can be obtained. Sampling techniques are difficult to devise, owing to the directional bias and agglomeration of damage, and very extensive counts have to be made if a true measure is to be obtained. When buds are pecked out by birds the damage is conspicuous but the effect of this damage on yield of fruit is obscure. In one attempt to measure this the damage to Conference pears was assessed by bud counts, at monthly intervals throughout the winter, and the subsequent crop was also recorded. The counts showed that individual trees had suffered damage varying between 6 and 90 % loss of buds, but on trees showing up to 70 % loss of buds there was no correlation between damage and yield. In order to estimate the effect of cumulative damage the yields from two plots of Leveller gooseberries, a variety in which fruit buds are not regenerated, were compared. One plot was situated near the edge of the plantation and had suffered bullfinch damage for about 5 years; the other plot was near the middle of the plantation where damage had never been known to occur. The undamaged bushes yielded an average of 16¾ lb. per bush, whilst the damaged bushes only averaged 5 lb. per bush. These two examples show the need for caution when assessing damage; they also demonstrate quite clearly that, while the effects are varible, bullfinch damage can severely reduce the crop.

It is an interesting feature of bullfinch damage that certain varieties are much more severely attacked than others. The birds' preferences differ locally and annually but dessert varieties generally suffer most damage. A few varieties seem resistant to attack, the pear Doyenné du Comice, for instance, rarely suffers any damage at all, although adjacent trees of other varieties may be stripped. The reason for this selectivity is not known, but it seems more likely to depend on the chemical constitution of the bud than on its morphological characters. It is also noticeable that bullfinch damage is most severe around the perimeter of orchards, especially adjacent to hedgerows or woodland. Owing to their shyness, these birds are reluctant to leave the cover and safety of their natural habitat for the comparatively exposed orchard. Thus they feed near the edge and retreat to the hedgerow at the first sign of danger. The removal of as much natural cover as possible often leads to a substantial reduction in damage.

It was at one time suggested that extensive damage occurred following the immigration of large numbers of bullfinches from the Continent. The northern bullfinch (*Pyrrhula p. pyrrhula* L.), which inhabits Scandinavia and the Baltic region, is an irregular visitor to

Scotland and north-east England, but is easily distinguishable from our native bullfinch by its larger size and brighter colour. The European bullfinch (*P. p. coccinea* Gmelin) closely resembles the British race (*P. p. nesa* Math & Ired.) and they would almost certainly be indistinguishable in the field. In the hand, however, they can be separated by a slight difference in wing measurement and in the hue and distribution of the breast colour. Harrison (1958) examined the bodies of over 600 bullfinches, shot in the orchards of south-east England, and concluded that they all belonged to the typically British race. The negative evidence provided by coastal bird observatories also supports the view that no large-scale immigration of bullfinches occurs. The data provided by ringing suggest that in this country bullfinches are very sedentary, recoveries usually being within 2 miles of the place of marking. On this evidence it seems reasonable to conclude that bud damage on fruit farms is done by locally bred bullfinches.

A study of bullfinch breeding has been made on a 350-acre farm, about half of which is devoted to fruit cultivation and the remainder to other crops or woodland. The principal objectives of this study were to determine the breeding habitat, the length of the breeding season and the breeding success. It was also intended that nestlings should be ringed for longevity and migration studies. The most common breeding sites were hawthorn bushes and brambles and the greatest number of nests was found where these occurred together, as in neglected hedgerows. Although well-trimmed hedges discourage bullfinch breeding, their nest site requirements are too unspecialized to make habitat control a practical method of reducing the population. The duration of the breeding season was ascertained by methodical searching of the whole farm (7 miles of hedgerow), at least once every 3 weeks, until no new nests could be found. The earliest eggs were laid towards the end of April and the last young to leave the nest did so in mid-September; the peak of breeding was reached in the first week of June with a subsidiary peak in late July. For practical purposes the breeding success was determined by the amount of predation; in four successive years 52 % ($\pm$ 3 %) of nests were destroyed by predators. The difficulty of finding nests and the extended breeding season make nest destruction an impracticable form of control for this species.

The skulking behaviour of bullfinches makes it extremely difficult to count these birds. The transect walk method proved useless and as the male does not hold a territory or sing, in the accepted sense of the term, dawn counts of singing males are not possible either. Nevertheless, in the course of frequent visits to the farm a general impression of the population size had been formed. It was of interest, therefore, to compare this subjective estimate with the number of breeding pairs located. In April 1957 the bullfinch population of the farm was estimated at between forty and fifty birds. Susbsequently fifteen concurrently occupied nests were found, indicating a minimum population of thirty birds. It cannot be claimed that every occupied nest was found but, in view of the thoroughness of the searching and supplementary observations on the behaviour of all bullfinches seen in the area, it can confidently be claimed that at least 50 % of all nests were located. Allowing for this possible error the maximum breeding population would have been sixty birds, so the original estimate seemed reasonable. Throughout the breeding season thirty-four occupied nests were found (first, second and perhaps third broods, including replacements following predation) and these produced forty-six young. At the end of the breeding season, therefore, the bullfinch population was between 76 and 152 birds. On an acreage basis this gives one bullfinch per 2½–5 acres. The number of bullfinches caught in subsequent trapping trials have led us to believe the higher figure to be the more correct. No allowance is made for post-fledgling mortality but the ratio of juveniles/adults in the trapped sample suggests that it is not high in this species in the period immediately after breeding. Whilst these figures apply strictly to the study area, they give some indication of the bullfinch population to be found in the weald of south-east England.

The natural food of bullfinches throughout the year falls into three categories, viz. annual weed seeds, tree seeds and buds. Annual weeds provide an ample supply of seeds throughout the summer and early autumn. When weed seeds become scarce the seeds of blackberry (*Rubus fructicosus*) and trees such as ash (*Fraximus*), alder (*Alnus*) and birch (*Betula*) are taken. As the supply of seeds eventually dwindles buds form an increasing proportion of the diet, and

from January to April bud eating is normal feeding behaviour. The buds of many wild trees are eaten but when fruit buds are available they seem to be preferred. This may be because they have a higher nutritive value than wild buds. Since small birds spend most of their time feeding, a knowledge of their feeding habits is a sure guide to their whereabouts at any particular season of the year. Such knowledge is essential if control measures are to be successful.

Growers who experience bullfinch damage usually protect their trees by shooting the birds. This is an expensive operation and if the birds are shot in the orchards it injures the trees. The best time to shoot is before the attack on fruit buds begins, when the bullfinches are feeding on tree seeds in the hedgerows and woodland. They are then less wary than at most other times and reveal their presence by constant calling. A better method of control, and the one that is now recommended, is trapping. The Protection of Birds Act, 1954, permits the use of a cage trap for the purpose of taking birds listed in the Second Schedule (harmful birds) and the bullfinch is included in this list in respect of certain specified fruit-growing areas. A traditional type of trap, the Chardonneret, is used but detailed improvements have been made to the trip mechanism to give trouble-free operation with a minimum of attention (Summers & Wright, 1959). The trap consists of a wire-netting box with a hinged lid. In the set position the lid is held open by a prop which engages with a horizontal perch inside the trap. When a bird enters the trap, and alights on the perch, the lid is released and falls shut. The traps are baited with seed heads of dock, the sharp dock (*Rumex conglomeratus*) and bloody veined dock (*R. sanguineus*) being the best. Using these baits and setting the traps in hedgerows or thickets, known to be frequented by bullfinches, few birds of other species are caught. Protected species can, in any case, be released unharmed. Fruit growers are finding traps more effective and economical than shooting and their use is spreading rapidly.

Other methods of preventing damage, which do not involve the destruction of the birds, have been tried but without much success. In particular much work has been done on chemical repellents, but the results achieved so far have been disappointing. With the exception of sticky substances, none of the chemical treatments applied to fruit trees has prevented damage by bullfinches. This does not necessarily mean they were not repellent, in some cases they may have been washed off by rain before the birds ate the buds. Sticky substances will deter birds but if they are applied in excess there is a danger that they will contaminate the birds' plumage, or actually catch very small birds. In these circumstances the substance may be deemed to be a bird-lime, the use of which is prohibited by the Protection of Birds Act, 1954. There is sufficient evidence to indicate that some species of birds can be repelled by the use of such chemicals as thiram and anthraquinone, but the conditions under which repellency occurs are not yet fully understood. Improved formulations containing 'stickers', applied at 'low-volume' rates, hold some promise of more favourable results and work is proceeding along these lines. The perfect bird repellent has yet to be discovered but the chemical treatment of crops may one day be developed to the point where a significant and economic reduction in bird damage can be achieved.

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POPULATION DYNAMICS OF THE WOOD-PIGEON (*COLUMBA P. PALUMBUS*) AND METHODS OF CONTROL

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If, for any reason, an animal becomes a pest it is usually desirable to exercise some form of control. To do this efficiently we have to base our methods on a thorough understanding of the animal's biology, i.e. its feeding and breeding habits, distribution and general behaviour. Only after studying the biology of the bullfinch (*Pyrrhula p. nesa*) was it found possible to control them by trapping (Wright, 1958). The rat's feeding behaviour is utilized in standard poisoning techniques of control. It is not sufficient to study the individual and the way it lives, it is also necessary to understand the special properties exhibited by populations. Information about the species numbers, density, population turnover and replacement rate is required because of the need to control populations, not individuals. Population ecology is a very recent science and every animal provides us with new problems of methodology. If, through an understanding of the above factors, it should prove possible to interfere with population balance by a control method, it remains to be shown that the cost of control is economic. In the case of many animals including the wood-pigeon, the animal is a pest because of its extreme abundance and efforts by many people are necessary in order to control it; this again introduces new problems.

It is now generally appreciated that animals are in some state of equilibrium with their environment. Although the breeding potential of animals is high, populations do not increase in size continuously. Some factor, or factors, limit this tendency and these may be density dependent, or independent (Nicholson 1933). Lack (1954) who has applied these considerations to birds, considers that food is the ultimate regulating factor.

These ideas imply that any reduction in wood-pigeon numbers brought about by artificial means would result in natural controlling factors being relaxed. The population would no longer be limited by its food supply and, left alone, would increase to its normal 'stable' equilibrium. Thus environmental changes would be needed to ensure a permanent reduction in numbers. There is evidence that the wood-pigeon originally lived in deciduous forests and depended on wild tree fruits and foliage for its food but also foraged on the ground in forest glades. In such habitats the species is fairly thinly distributed. Deforestation and the subsequent increase in arable farming in this country has created a new environment within the last 100 years. Fields are planted with suitable food (grain, legumes, clovers) and these can be regarded as being extensions of the original forest glades. At the same time small areas of woodland are preserved as isolated copses and hedgerows and these provide abundant nesting cover. The wood-pigeon has consequently increased in numbers with the increase in food supply and has become a pest. We cannot contemplate controlling the wood-pigeon by altering the present environment—so control measures must be applied to the bird itself. Furthermore, since a policy of extinction would not be realistic, *repeated* control is necessary.

Many methods have been employed to count birds and to measure population changes, but there are few species for which much information exists. The most satisfactory method of study involves having a marked population. Marking itself presents many problems, and a technique which is of use for one species may not be applicable to another. The wood-pigeon is a particularly difficult subject; its wariness makes close observation a problem; it ranges over a wide area in its daily routines; the sexes are identical in appearance and juveniles can only be distinguished from adults in the field for a short period. The following techniques for studying wood-pigeons are either in use or are contemplated: *Direct counts* can be made as for some other species, for example, rooks and herons nest in a conspicuous way and are easily counted during their breeding season; aerial photographs have been taken of geese flocks in winter; the singing males of some territorial species may also be easy to

count. Fisher (1952) has used direct counts of fulmars and other sea birds, on their nesting ledges on sea cliffs, to enable him to measure world changes in population size. The most useful method of this sort for wood-pigeons is to count them as they leave their roosts in the morning. This can only be done during the period November–March. In this way the total population of a large area can be calculated. It is impossible to employ direct counting during the summer months as the birds do not then congregate in flocks. To estimate the size of a breeding population it is possible to assess the *occupied nest density* (Murton, 1958). Although of little use for many species, this method is possible for wood-pigeons owing to the ease with which nests can be found. For total population counts by this method allowance has to be made for the proportion of non-breeding birds, or pairs which have lost a nest or which are only preparing to lay eggs. This is arrived at by examining samples of shot birds to determine the proportion not in reproductive condition and in addition direct watches are made in sample areas within the nesting places, to estimate the number of birds preparing to nest. Counts are also made of singing males to discover the number of established territories. Although subject to certain errors the method is of great use for comparative studies. *Capture–recapture techniques*, such as the Lincoln Index method of estimating population size have been widely used in the study of small mammals, but they have not yet been applied to wood-pigeons owing to the difficulty of catching them.

Some indication of seasonal and annual changes in the population structure could be derived from the ratio of juveniles to adults if random samples of a population could be obtained. In this way the effect of control techniques might be measurable without having a marked population. Experiments are in progress using narcotized baits to catch wood-pigeons and any captures will also be used for this purpose.

The national bird-ringing scheme, which is administered by the British Trust for Ornithology, provides capture–recapture data of a special kind. Under this scheme birds have been ringed throughout the British Isles by a large number of amateur ornithologists since about 1900. Many birds are ringed as nestlings so that on recovery their ages are known. It is possible to construct life tables from these data and to estimate average survival rates (see, for examples, Lack, 1943; Haldane, 1955). This has been done for the wood-pigeon and shows that the average annual mortality of adults is between 30 and 40 %. However, in order to measure annual variations in mortality and to follow the effects of applying control measures, techniques such as those described previously are still necessary.

At this point it is useful to discuss age in relation to control. As mentioned earlier we know that reproductive capacity is not the factor limiting the size of healthy populations. If all young survived, population size would increase by geometrical progression. This does not happen because usually juvenile mortality is proportionately higher than that of adults. For this reason, many workers doubt the value of control techniques that destroy primarily juveniles, because many will die in any case; however, they often live long enough to cause damage. Juveniles, when they leave their nests in August and September, find much grain on the stubbles. It is not until December that food really becomes scarce. If hard weather occurs in December and January and snow covers the ground very little food is available. It is at this time that wood-pigeons descend on to the tall standing crops, such as brassicae, and cause immense damage. During the autumn, when food is abundant, adults accumulate considerable fat deposits but the juveniles which are still growing do not. If hard weather continues it is the juveniles which are the first to suffer. These data come from weighing and examining samples of birds shot throughout the year. Wood-pigeon shooting does not start in earnest until the end of the pheasant shooting season, as before this time estate owners are reluctant to allow ready access to their coverts. A peak in the number of wood-pigeons shot thus occurs in February, when the organized shoots, sponsored by the Ministry of Agriculture, come into operation. A study of shot birds (including those ringed) shows that, up to this time at least, proportionately more juveniles are killed than adults. This is because juveniles are less cautious than adults. Although there is no objection to killing young, because they also do damage, far more good would be done by concentrating the shooting programme in October instead of in February, but it is difficult to see how sporting traditions could be changed to this end.

At present the costs of nest destruction and shooting can be estimated, and these costs have been compared with each other. The method of controlling wood-pigeons by nest destruction has recently received much publicity and is now recognized as a supplement to shooting (Murton, 1959). In certain circumstances the destruction of wood-pigeon nests may qualify for a grant, from the Ministry of Agriculture, of up to half the cost incurred. A full description of the method, together with experimental results, has appeared in this journal (Murton, 1960). As wood-pigeons are so numerous the success of this technique, as of many others, depends on the co-operation of a large number of people, mainly farmers.

To determine what support would be given to nest destruction as a method of control a survey was carried out among 442 occupiers of land in East Anglia. From groups of occupiers of land of over 1 acre, about fifty were selected at random in each county. Their practical co-operation was sought by sending each occupier a leaflet describing how, when and where to undertake nest destruction and pointing out the advantages of the method. Subsequently, the local Pests Officer visited the occupier three times and wrote down the answers to a standard questionnaire. The interviews took place during the following periods: 23 June–14 July, i.e. before nest destruction should be done for the first time; 25 August–8 September, i.e. between the two nest-destruction periods, and 22 September–6 October, when all work should have terminated. Most occupiers had read and understood the leaflets and answered the question put to them.

Of 442 people interviewed, 94·3 % considered that wood-pigeons were a serious pest to agriculture, 86·9 % said they had wood-pigeon problems on their land, 59·7 % were not satisfied with present control methods and 65·2 % said they would welcome a new control method. Very few people objected to nest destruction as a control method and 84·4 % promised to take part in the first trial.

TABLE 1. *Number of people interviewed in each county promising and actually carrying out nest destruction* (N.D.), 1958

County and size of original sample	No. promising to do 1st N.D.			No. who did 1st N.D.		No. promising to do 2nd N.D.			No. who did 2nd N.D.		No. promising to do three N.D.'s in future		
	Yes	No	Don't know	Yes	No	Yes	No	Don't know	Yes	No	Yes	No	Don't know
Beds. 24	21	2	1	13	11	5	7	12	9	15	4	18	1(†)
Cambs. 48	46	2	0	39	9	46	2	0	20	28	46	1	1
Essex 43	34	8	1	19	24	17	21	4*	6	35*	15	12	14
Hunts. 48	29	11	8	29	19	24	9	15	9	39	34	2	12
Isle of Ely 24	23	1	0	16	8	21	3	0	8	16	18	4	2
Lincs. (Holland) 64	56	3	5	49	15	53	3	8	49	14(†)	51	1	10(2†)
Lincs. (Lindsey) 46	43	3	0	37	9	43	3	0	26	20	41	3	0(2†)
Norfolk 49	47	1	1	38	10	40	3	5	18	31	37	3	7(†)
Suffolk 45	31	5	9	15	30	8	7	30	7	38	5	12	28
Total	330	36	25	255	135	257	58	74	152	236(†)	251	56	75(6†)
		391		390			389		388			382	
%	84·4	9·2	6·4	65·4	34·6	66·1	14·9	19·0	39·2	60·8	65·7	14·7	19·6

Notes. The original sample size of 391 was not maintained at each interview, because either no answer was given (denoted by † above) or an interview was impossible owing to unco-operation. (This is shown above by an asterisk and denotes that one occupier dropped out of the sample.)

Since the properties were randomly selected, some of them unavoidably contained no nesting sites and these had to be discarded. This left 390 occupiers eligible to undertake the first period of nest destruction and 65·4 % did so (Table 1). For the second nest destruction of 388 eligible, 39·2 % took part. Two nest destructions were carried out by 36·3 % of the occupiers. It was also possible to determine the percentage acreage of land covered by two destructions. Altogether 69,007 acres, distributed among 381 occupiers with nesting sites, were sampled. Nest destruction was done twice in 24,256 acres so that 35·2 % of the area was covered. At the same time a test was made to determine whether the support for nest destruction varied with the size of the property but no statistically significant differences were found.

The percentage of occupiers who considered the wood-pigeon to be a problem varied in each county. Reference to Table 1 shows that the proportion undertaking the trials also varied per county. When these two variables were plotted against each other it was apparent that in those counties where farmers were most concerned about the wood-pigeon problem, a larger proportion actively supported our trials (a correlation test was not quite significant). This illustrates the difficulties, which have to be overcome, in introducing control techniques when local opinion does not believe them necessary.

As already stated 36·3 % of those occupiers with nesting sites carried out two nest destructions. This figure must be modified depending on how well they did the operation. Of the 142 who did two nest destructions about sixteen did them at the wrong time, therefore, the efficiency of the trials becomes about 32 % based on 391 eligible interviews. Allowing for a further reduction in efficiency due to the fact that not all the property was covered, the approximate figure for the trials is about 30 %. This figure is a fair guide to the amount of practical support given to one control method. It does not represent the public feeling in favour of nest destruction which, of course, is a much higher percentage.

Knowing the efficiency with which one control method is carried out in practice, it is possible to relate this to the efficiency of controlling the pest. With two periods of nest destruction approximately 63 % of the young which would leave their nests are prevented from doing so (Murton, 1960). Our studies of wood-pigeon populations have shown that 100 adults produce roughly 100 young, which leave their nests, during the breeding season. With two nest destructions only 37 young in every 100 would leave their nests successfully. If two nest destructions are carried out with only a 30 % efficiency then of 100 young only 18·9 (30 of 63 %) are prevented from leaving their nests. Of course, a high proportion of these young die before becoming adults and, as this mortality is density dependent, the number dying would vary depending on whether control had been carried out or not. Done as recommended, nest destruction prevents about half the potential adult replacement; whereas done as in these trials only one-fifth of this replacement is affected.

It is apparent that nest destruction, although fairly efficient when carried out as recommended, is much less efficient when put into practice on a large scale. The same situation would probably apply to any other control technique that depended on action at a specific time by many people. This suggests that the best forms of control would be those depending on as few people as possible. Enough has been said to indicate that even if control methods for the wood-pigeon are biologically satisfactory, their execution is subject to considerable variation, and at this point a scientific approach, perhaps analogous to modern consumer research, is necessary.

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THE BIOASSAY OF RESIDUES OF INSECTICIDES IN SOIL

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Introduction

The use of insecticides in soil is an old and well-established practice for controlling certain subterranean pests such as wireworms and leatherjackets. However, during the past decade, the practice has been extending rapidly as more species of soil-inhabiting pests are being controlled by an increasing range of insecticides. For instance, the carrot fly, *Psila rosae* F., can now be readily controlled by mixing aldrin or dieldrin into the top few inches of soil just before sowing the crop (Wright, 1959) and diazinon used in a similar way can successfully reduce populations of the lettuce-root aphid, *Pemphigus bursarius* L. (Dunn, 1960). Unfortunately, many insecticides persist and remain active for several years when they are mixed with soil (Fleming & Maines, 1953; Wright, 1959) and, if treatments are repeated frequently, problems may arise concerning the possible accumulation of residues.

The strict legal tolerances governing the residues of pesticides permissible in marketed produce in the United States of America have, in recent years, stimulated interest there in the subject of residues in plant and animal tissues and to a lesser extent in soils. At present, most of our knowledge of methods of assay and of the fate of insecticides in soil originates from work done in the U.S.A.

That the use of insecticides in soil may also create problems here is well demonstrated by the current experiments of Mr D. W. Wright on the control of the carrot fly. In one experiment he has shown that dieldrin applied to a fen soil in March, 1956, at doses of 2 and 4 lb./acre still gave 80 and 98 % reduction of a carrot fly population, respectively, in 1959, four seasons later, indicating that this material is extremely persistent when mixed with soil. In the same experiment the rates of loss of aldrin and lindane were more rapid than for dieldrin, but their residues were apparently more effective against the pest in 1959 than in 1958 although, obviously, larger residues must have been present in 1958 than in 1959. Clearly there is much yet to be learnt about the use of insecticides in soil and, if more detailed information is required on their persistence in this medium, it is necessary to be able to assay with reasonable accuracy the levels of residues likely to be encountered in the field. Either *chemical* or *biological* methods of assay may be used but our concern at the moment is with the latter, the so-called bioassay techniques.

Methods of bioassay

The principles upon which bioassay methods are based and the factors which govern the choice of test organisms have been discussed in detail by Sun & Sun (1952), Sun & Pankaskie (1954) and Dewey (1958). Two main types of technique have been used for the bioassay of insecticide residues in soils, these being the direct and the indirect or extraction methods. Fleming & Maines (1953), Lange & Carlson (1955) and Wylie (1956) exposed the test insects directly to the soils containing the residues. Because of their relative simplicity, these direct methods are attractive but they are of limited sensitivity and are mainly employed when residues exceed 0·5–1 p.p.m. Fleming, Coles & Maines (1951) found excessive variation in results obtained by a direct method and they resorted to an extraction method both to reduce variability and also to improve sensitivity. Extraction methods have also been used by Terriere & Ingalsbe (1953), Berger & Randolph (1958) and Kiigemagi, Morrison, Roberts & Bollen (1958) and a number of different solvent systems have been employed to extract the insecticide from the soils. The extraction techniques are more elaborate than are the direct methods, but they do offer a considerable improvement in sensitivity at the expense of introducing as a possible complication the variable efficiency of an extraction process

(Lichtenstein & Schultz, 1958). A compromise method of intermediate sensitivity has been evolved by Edwards, Beck & Lichtenstein (1957) who placed a small aliquot of the soil sample into a test jar and then added sufficient of a volatile solvent to form a slurry. The solvent was allowed to evaporate and apple sauce was added to the surface of the soil to attract the test insect, *Drosophila*.

The sensitivity required

The choice of the method of assay is governed to a considerable extent by the range of concentration of insecticide likely to be met in soil and, especially, by the lowest concentration which will have to be assayed. In this country, amounts in excess of 5 lb./acre are unlikely to be applied other than on experimental plots, so that the maximum concentrations to be handled would not be expected to exceed the range 5–50 p.p.m. depending on the depth of incorporation. Doses of 1–3 lb./acre, mixed initially to a depth of about 4 in., are commonly used in practice but subsequent ploughing to a depth of, say, 8 in. would further dilute the material. Thus concentrations of 0·5–3 p.p.m. would be frequently encountered (1 lb./4 in. deep acre ~ 1 p.p.m.). If it is desired to follow the fate of the insecticide until only about 10% of the original remains, then the method must be able to assay 1/10 of the smallest dose likely to be used, mixed to the maximum probable depth of cultivation. Therefore, ideally, a technique should be able to assay the commonly used insecticides at concentrations of about 0·03–0·05 p.p.m. in soil, i.e. 0·1 lb./acre mixed to a depth of 8–12 in.

The development of a sensitive technique

In connexion with work on the control of the carrot fly, attentions were directed initially to devising a bioassay technique which would be satisfactory for dieldrin residues at concentrations of 0·05–5 p.p.m. in soil. The method described by Edwards *et al.* (1957) appeared to warrant further investigation to see whether its sensitivity could be inproved. However, no means of markedly improving the sensitivity was found and the minimum concentrations of dieldrin which could be assayed were about 0·15 p.p.m. in sandy soil and 0·5 p.p.m. in highly organic fen soil, minima considered inadequate for our purposes. In addition, the results were rarely consistent and variations in the texture of the soil in the jars appeared to be important in this respect. When the air-dried soil was ground in a small laboratory mill, the variability was much reduced but, under certain conditions, the air-drying process resulted in an almost complete loss of small residues of both aldrin and dieldrin. It was concluded, therefore, that only an extraction technique would be likely to prove sufficiently sensitive.

Lichtenstein & Schultz (1958) described a tumbling method for extracting insecticides from soil and a modification of this method, using acetone as the solvent, has been found to be satisfactory for the present purpose. An aliquot of the soil sample and a known volume of acetone were tumbled end-over-end for 1 hr. in a sealed bottle to enable the insecticide present to reach an equilibrium concentration in the solvent. The suspension was allowed to settle and the supernatant liquid was withdrawn to provide an extract containing the insecticide and other extractives in a mixture of acetone and soil water. Untreated soil was used to prepare control extracts and assay standards were made by adding known amounts of the insecticide to untreated soil in the extract bottle *before* tumbling.

Adults of *Drosophila melanogaster* Meig. were used as test insects and were exposed to deposits obtained by applying aliquots of the extracts to cotton dental rolls (38 × 10 mm. diam. approximately) and allowing the solvent to evaporate. As was to be expected from the dose/mortality relationships for *Drosophila*, only a limited range of doses could be assayed directly for any fixed time of exposure. This difficulty is common to nearly all dose/mortality methods and it often means that effort must be wasted in finding the approximate level of residues present in unknowns before accurate assays can be done. As little as 0·1 μg. dieldrin/roll could be measured, however, if sufficient time was allowed for the flies to respond and thus the range could be increased considerably by assessing the response of the flies after different times of exposure. This procedure led logically to time/mortality data

and to the principle of the method reported by Callaway, Dirnhuber & Wilson (1952). The time for 50 % of the flies to respond could be estimated and related to the dose, and by this means it was possible to cover directly most of the range of dieldrin residues in soil likely to be encountered in practice, namely 0·03–5 p.p.m.

Two practical difficulties arose with this time/mortality method. First, a logarithmic time sequence of observations was required and, when the exposure times were short, it was often physically impossible to complete one set of records before the next was due. Secondly, the records had to be continued throughout the 24 hr. for one or more days which resulted in considerable human strain. In order to overcome these difficulties, an apparatus has been constructed to record automatically the response of the flies (Wheatley & Hardman, 1960).

Residues of dieldrin in a sandy loam soil

The technique outlined above has been used to study the effect of cultivation on the depth distribution of dieldrin in two plots on a sandy loam soil at Wellesbourne. The dieldrin had been applied to the surface of the soil on the first plot in April 1959, at 12 oz./acre and had been mixed in to a depth of 10 cm. by a double rotary cultivation. Soil samples were obtained in September 1959, using a 26·7 mm. diameter core sampling tool, and ten cores were removed intact and cut into 1 cm. layers to a depth of 9 cm. and thereafter into 3 cm.

TABLE 1. *The influence of cultivation on the depth distribution of dieldrin in a sandy loam soil*

Dose	...	12 oz./acre*	32 oz./acre
Applied	...	Apr. 1959	Mar. 1956
Assayed	...	Sept. 1959	Nov. 1959
Cultivations	...	Rotary, 4 in.	3 ploughings, 8 in.
Depth (cm.)	...	(oz./acre)	(oz./acre)
0–1		1·1	
1–2		1·0	4·0
2–3		1·3	
3–4		1·0	
4–5		0·9	3·1
5–6		0·6	
6–7		0·2	
7–8		0·0	3·5
8–9		0·0	
9–12		0·0	3·8
12–15		0·0	3·4
15–18		0·0	2·2
18–21		0·0	1·4
21–24		0·0	0·3
24–27		—	Trace?
27–30		—	0·0
Total		6·1	22

* An assay done immediately after treating this plot showed that only 9 oz. dieldrin/acre was present. The reason for the discrepancy between the amounts applied and recovered is at present obscure.

layers to 24 cm. The comparable layers from each core were thoroughly mixed by sieving to provide samples for the assay. The results (Table 1) show that no insecticide was apparently present below 7 cm. indicating that it had not been mixed to the full depth of cultivation. This confirms the findings of Grainger (1958) who has used ^{131}Iodine to examine the mixing efficiency of various methods of cultivation.

In the second plot examined, dieldrin had been applied at 32 oz./acre in March 1956 but, in addition to two initial rotary cultivations, the plot had been ploughed to a depth of approximately 20 cm. on three subsequent occasions. In November 1959, twenty cores

were removed and sliced into 3 cm. layers to a total depth of 30 cm. and the samples for each depth were pooled and assayed. The results shown in Table 1 indicate that ploughing had been effective in distributing the insecticide to the full depth of cultivation.

The slow decline of dieldrin in a sandy loam soil at Wellesbourne was shown by assaying the residues in plots which had been treated with dieldrin at 1, 2 and 3 lb./acre in March 1956. The doses were replicated twice in the field and duplicate samples, each of fifty cores, were obtained from each plot in November 1959. Duplicate extracts were prepared from each sample and two estimations were made on each extract. The results are given in Table 2 and they suggest that the half-life of dieldrin in this sandy loam soil was approximately 4 years. For the analysis of variance, the logarithms of the estimated residues were used, in order to equalize the variance for the different residue levels and, hence, the standard error for a plot is given as $\overset{\times}{\div}$ by a factor (1·07 ~ ±7%) rather than as ± a quantity. The amounts lost, expressed as percentages of the amounts applied, are also shown in Table 2 and they indicate that the rate of loss of dieldrin may vary inversely with the amount present, as found by Lichtenstein & Schultz (1959*b*), but, to be certain, more accurate estimations than these would be needed.

TABLE 2. *Residues of dieldrin (lb./acre) remaining in November* 1959

lb./acre, applied March 1956...	1	2	3
Block			
I	0·46	1·1	1·8
II	0·49	1·2	2·1
Mean % loss	53	45	37

Standard error for a plot; $\overset{\times}{\div}$1·07.

Comparison of results obtained by chemical and biological methods of assay

Lichtenstein & Schultz (1959*a*) have postulated that complete agreement between the results of chemical and biological methods of assay could be expected only in restricted cases. They argue convincingly that agreement between the methods will depend upon the biological and chemical properties of the breakdown products of the original insecticidal compound. If an insecticide 'A' gives rise to compound 'B', the latter may or may not be either toxic biologically or reactive chemically which would lead to four possible combinations in the results, without considering other complications which could occur. Only if 'B' is both non-toxic and unreactive would the two methods be expected to agree. In all other combinations of toxicity and reactivity, discrepancies between the methods are to be expected and, indeed, are met with in practice. Lichtenstein & Schultz (1959*a*) conclude that both methods are complementary, but that results by bioassay are inclined to agree better with the practical importance of the residues.

Bioassay methods are relatively simple and can be highly sensitive but, because they are non-specific, the advantages offered by these techniques cannot be fully utilized at present. The need for equally straightforward methods of identifying insecticides is very evident and it seems probable that partition chromatography will be used increasingly for this purpose.

We are indebted to Mr D. W. Wright for the interest which he has shown in this work and also for permission to quote from his unpublished results on carrot fly control and to make use of his experiments for the residue studies. The invaluable technical assistance of Miss G. D. Bubb and Miss C. M. Lowe is also gratefully acknowledged.

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A TRANSMISSIBLE DISEASE OF CULTIVATED MUSHROOMS ('WATERY STIPE')

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'Watery Stipe' is one of three names given to a type of mushroom disorder which is of considerable economic importance to the industry. The other two are 'La France disease' and 'Brown disease'. There has been some controversy over the question of whether three separate disorders exist, or whether they are simply different manifestations of the same one.

'La France disease' was first described by Sinden & Hauser in 1950, but very briefly, which made the description of little use for the identification of the disease by others. Sinden identified 'La France disease' in the United Kingdom 2 or 3 years later and sporadic outbreaks of suspected 'La France' were reported up to 1957. In that year, however, an unidentified disorder spread to most parts of the country where mushrooms were grown

and reached epidemic proportions. As the symptoms differed slightly from those described by Sinden the disorder became known firstly as 'Brown disease' and later 'Watery Stipe'. As might be expected the symptoms observed in outbreaks on commercial farms varied, but can be classified into four groups:

(1) Much reduced cropping. At the height of the 1957 outbreaks crops were down to a quarter or less of normal.

(2) A large proportion of the sporophores which do develop die whilst immature, thus decreasing the crop still further. Environmental conditions seem to influence considerably the general appearance of such sporophores.

(3) The production of abnormally shaped sporophores. The most usual deformity is an elongation of the stipe, which is also often bent at right angles. These elongated sporophores usually have small pilei and are aptly described as 'drum-sticks'.

(4) The watery stipe symptom is caused by waterlogging of the sporophore tissues, which appears usually as translucent longitudinal streaks on the stipes. These streaks frequently enclose a cavity in the tissue.

It is obvious from a survey of several outbreaks that no single symptom can be regarded as diagnostic. Thus, identification of 'Watery Stipe' has been based upon an assessment of these different symptoms.

Two theories were developed as to the cause of 'Watery Stipe', both based largely upon observation of affected crops in 1957. One was that it was caused by environmental conditions, in particular high temperature and humidity, and insufficient ventilation; it was shown that outbreaks were most frequent during periods of warm humid weather when ventilation in mushroom houses was most likely to be unsatisfactory. The other view was that 'Watery Stipe' was a pathological condition as it often appeared first in a restricted area, then spread to the rest of the house and finally to the whole of the farm. No improvement could be obtained by increased ventilation, but if the farm were cleared and disinfected the trouble disappeared. These two views are not altogether incompatible because if a pathogen is present, its behaviour and the type of symptom could be greatly influenced by the environment.

The investigation of 'Watery Stipe' at the Glasshouse Crops Research Institute began with the object of determining whether the condition was transmissible. Casing and compost were obtained from nine crops suspected of having the disorder. Owing to the difficulty of obtaining suitable material when required, only one importation of material has been made so far, the material for later experiments being obtained by saving compost and casing from the preceding one.

In the first series of experiments a comparison was made between the effect of inoculating freshly spawned trays of compost with (1) the material mentioned above and (2) water extracts of this material, the extracts being filtered to remove micro-organisms larger than yeasts and fragments of mycelium. The incorporation of BHC at the rate of 50 p.p.m. into the compost prior to filling, controlled the multiplication of pests introduced with the material. Periodic sampling of the plots showed that the numbers of pests present were well below the danger level.

The results of the first series of experiments were striking, the control and water extract plots giving similar yields, in contrast to the plots inoculated with material which yielded one-third of the number and one-fifth of the weight of the controls. Mycelium in the plots inoculated with material began to degenerate in some instances within 2 weeks of the first picking and in all had completely disappeared by the end of the experiment. There was no corresponding deterioration in the water extract or control plots.

The inoculation of plots with material has been repeated several times, with similar results. There have been no differences in the behaviour of the material from different sources. Although the mushrooms produced in the treated plots were often of poor quality, they were so few that it was impossible to estimate the frequency with which the macroscopic symptoms described earlier occurred. Abnormal mushrooms were found also on control plots, indicating either that the disorder had spread or that they were caused by environmental conditions.

The plots (6 sq.ft.) were too small to study rates of spread, if any. More recently, when shelf beds 12 × 4 ft. were used, no mushrooms were produced within a radius of about 3 ft. from the site of the inoculation. Beyond this area one true flush of mushrooms was produced and thereafter only scattered sporophores of very poor quality, but there was no marked increase in the size of the bare area. It is possible therefore, that the disorder spreads most rapidly when the mushroom mycelium is growing most actively, but that when the substrate has been fully colonized and fruiting begins, there is little further spread. If this is so, the severity of an outbreak would be related to the time of infection, which may explain some of the contradictory statements which have been made about the behaviour of the disorder, particularly rates of spread. Further experiments are planned to elucidate this point.

During all the experiments mycelial isolations were made at intervals and plated out on 2½% malt agar. In many instances the mycelium was in a poor condition and failed to regenerate, but the cultures which were obtained were of two types; (1) normal; producing white, fluffy mycelium and thick rhizomorphs radiating from the point of inoculation; and (2) an abnormal type: in which the mycelium was adpressed, without rhizomorphs, frequently buff coloured and very slow growing.

A series of isolations from plots showed that before inoculation the mycelium produced normal colonies on agar, but usually after inoculation the mycelium would eventually yield the abnormal type of culture, even though it had not shown visible signs of deterioration. Even more interesting results were obtained from plots in which the mushrooms showed a gradation from healthy to dead. Mycelium from the areas with healthy mushrooms produced healthy cultures, but those from areas with dead mushrooms produced abnormal growth, and sometimes these isolations were made from areas only 6 in. apart. Isolations from control plots, which had produced much heavier crops, showed no signs of degeneration.

The abnormal cultures differed from the normal ones microscopically. The mycelium was much sparser, especially the aerial part, and the individual cells were sometimes slightly swollen, giving the mycelium a somewhat gnarled appearance. Fewer calcium oxalate crystals were produced, so the mycelium lacked the furry appearance of the healthy one. In neither stained or living mycelium could any contaminant fungi or bacteria be found. It was reported in a private communication that some mushroom strains may be contaminated by a parasitic bacterium but that the culture could be cleaned by treatment with rose bengal. Treatment of the abnormal cultures with rose bengal at several strengths failed to produce any improvement in growth. Growth was also consistently poor on media of different richness. Inoculation into beef broth with abnormal mycelium gave no indication of the presence of bacteria.

If two healthy compatible cultures were inoculated on to an agar plate, the mycelia met and the rhizomorphs anastomosed. But when a normal and an abnormal culture were inoculated on to the same plate, the mycelia were slow to touch, a clear zone remaining between them for some time. The growth of the normal colony which took place subsequent to the touching of the two colonies was often weaker than usual, although no apparent change occurred in the appearance of the older mycelium. But when this older mycelium was removed and grown in isolation, its new growth was slow and abnormal. By isolating mycelium from different parts of the colony, it was possible to trace the spread of the infection throughout the mycelium. The transmission of the slow-growing condition to the healthy cultures has been demonstrated repeatedly in these experiments. This technique is now being employed for the identification of 'Watery Stipe' outbreaks; its greatest drawback is slowness, about 4 weeks being needed to complete the whole process.

Transmission to normal cultures has been obtained also by macerating abnormal mycelium in sterile water and dipping disks of normal mycelium in this suspension for a few seconds before plating out. If the liquid is filtered to remove the fragments of mycelium, infectivity is lost. This confirms the results obtained with water extracts of material in the first series of experiments in beds. The extracts so far used have contained fairly large fragments of mycelium which were capable of regeneration. It remains to be seen whether much finer suspensions will give the same results.

The experiments described so far were done at a temperature of 25° C., At 33° C. there

was a marked difference in the response of normal and abnormal cultures. With normal cultures, growth rate was slowed, no rhizomorphs were produced, and the colony was much denser than at 25° C. However, the growth rate of most abnormal cultures at 33° C. was increased and the mycelium became white and fluffy, so that it was indistinguishable from the normal mycelium grown at the same temperature. When young mycelium from these high temperature colonies was subcultured and incubated at 25° C., the normal colonies reverted to the original type of growth, but the abnormal colonies did not, and instead produced normal fluffy white mycelium. When tested by the double-inoculation method, these cultures failed to transmit any disorder. Very occasionally, heat treatment failed to improve the growth of abnormal cultures. These continued to give a positive reaction to the transmission test. Also the original inoculum in the heat-treated abnormal cultures remained infected. When abnormal mycelium was killed by heat, infectivity was lost.

This investigation is still far from complete so it is not possible to do more than hazard a guess as to the cause of 'Watery Stipe'. Lindberg (1959) described a disease of *Helminthosporium victoriae* which caused abnormal growth of this fungus and which was transmissible to normal strains. He has not so far been able to discover the cause of the disease. The similarity between the two diseases is so striking that one is tempted to think that the cause in both cases is similar. One of the main objects of future work will be to try to answer a question which has long been in the minds of many mushroom growers, namely, is it a virus?

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CECIDOMYIIDAE AS PESTS OF CULTIVATED MUSHROOMS

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Since its discovery by Wagner in 1862, paedogenesis (i.e. reproduction by immature forms) has been regarded as little more than a biological curiosity by entomologists. It is only in recent years that this phenomenon has assumed any degree of economic importance. The first reference to Cecidomyiidae attacking cultivated mushrooms was by Barnes (1926) and soon afterwards (Theobald & Barnes, 1928) paedogenesis was first recognized in a mushroom-infesting species. With the rapid post-war expansion of indoor mushroom cultivation, the Cecidomyiidae have suddenly come to the fore as major pests of mushrooms. In many higher plants, intensive cultivation brings with it ideal conditions for the rapid multiplication of pests, and similarly some mycophilous species are favoured by modern methods of mushroom culture.

Prepared mushroom compost is placed in trays or on shelves in a mushroom house and the whole is then thoroughly pasteurized at 130° F. This treatment not only completes the composting process but effectively eliminates all insect life. Most growers imagine that this is a good safeguard against insect pests being introduced from the compost stack. However, we have been unable to find any of the regular mushroom pests either in the fresh compost or in the original horse manure. After pasteurization mushroom spawn is introduced to the beds and the mycelium begins to run through the compost. It is apparently the smell of this rapidly growing mycelium which attracts the majority of Dipterous pests to the mushroom house. A few cecid flies can be caught in light traps at this time. On most commercial holdings several houses are cropped consecutively and throughout the year. Thus there is

a continuous source of re-infestation by flies. Also larvae can be transferred to a new house on clothing or dirty implements, unless elaborate precautions are applied.

By whatever means a cecid finds its way to freshly growing mushroom mycelium, the larva at once starts paedogenetic reproduction. The ovaries develop in the young larva and embryos are released and grow within the body cavity. In *Heteropeza pygmaea* Winn., the commonest species, an average of fourteen young emerge from the skin of the mother larva within 8 days under laboratory conditions, but in mushroom beds, about seven larvae are produced in 13 days. This reproductive rate continues unimpaired as long as adequate nutritious mycelium is available, that is, in a normal mushroom crop for 9 or 10 weeks from the time of spawning. By then the mushrooms have cropped for 4 weeks and most of the yield has been obtained. During this time the population grows exactly logarithmically, every introduced larva producing some 60,000 descendants.

In the light of recent knowledge of population dynamics, this would appear to be a surprising statement. Logarithmic population growth is a theoretical ideal but it is usually held that it never occurs in nature. Paedogenetic cecids are peculiar, however, in several respects, for they are unusual pests attacking a unique host. First, the peculiar mode of reproduction enables continuous existence in a favourable environment without a vulnerable dispersive stage. Secondly, mushroom cecids are not known to have any predators, parasites or diseases. Thirdly, the physical environment is kept remarkably constant by controlled heating and regular watering, so that climatic conditions are never limiting. Fourthly, the cecids find themselves in what is virtually a biological vacuum apart from a vast supply of their own food material; and, finally, mushroom mycelium being a continuously extending and anastomosing network does not appear to suffer, as would a more highly organized plant, from the attacks of innumerable larvae. Thus even intraspecific competition does not hinder exponential population growth, for an unlimited supply of food appears to be available over the 9-week period.

This latter statement must be substantiated, and it is here that we come up against one of the most unusual aspects of this problem. Three years ago work started on mushroom pests at the Glasshouse Crops Research Institute, and at that time many mushroom growers were complaining of serious crop losses, or even crop failures due to cecids. However, in a series of eleven cropping experiments we have been unable to demonstrate any decrease in the weight of mushroom crops due to cecid larvae, even when present in very large numbers. The mushroom physiology is not upset by the heaviest infestations, and one is led to conclude that ample nutrients are available both for the production of mushrooms and the feeding of innumerable pests.

After about 9 weeks the mushroom mycelium appears to undergo a change, possibly associated with the fall-off in cropping. At this stage the mycelium is no longer suitable for the rapid increase of cecids and the population stabilizes and many start to fall. At about this time the behaviour of the larvae alters in several ways. They frequently move from the mushroom beds in vast numbers and congregate in masses on the floor. Also many pupate and produce adult flies which may infest new crops. Yet others form a toughened brown cuticle and produce one or two daughter larvae which can remain protected within the mother for at least 18 months. Our understanding of these terminating processes is still very incomplete; the production of 'resting mothers' is associated with partial starvation, but the factors determining pupation and migration have yet to be investigated.

So far the account has been illustrated by reference to one species, *Heteropeza pygmaea*. The other species involved belong to two subfamilies: the Heteropezinae and the Lestremiinae. The Heteropezinae include *H. pygmaea* and a newly discovered species *Henria psalliotae* Wyatt. The latter species is most unusual as it is paedogenetic in the pupal stage and can produce as many as ninety-six young. It has been recorded only once, from Cornwall, and is unlikely to be a serious pest. To the Lestremiinae belong *Mycophila speyeri* Barnes and *M. barnesi* Edwards. *M. speyeri* is probably a greater threat to mushroom growers than is *Heteropeza pygmaea* for its reproductive rate is considerably higher. It produces about twenty young in 6 days under laboratory conditions, compared with *H. pygmaea*'s fourteen young in the same period.

Thus it is able to reach peak populations by the beginning of cropping whereas *H. pygmaea* does not reach the same population until near the end of cropping. It is not certain why the population of *M. speyeri* declines soon after cropping begins, for it cannot be influenced by the same physiological change in the mycelium which appears to check the population growth of *H. pygmaea*. It is possible that population pressure is here taking effect. In population studies growth trends of *M. speyeri* are not as predictable as those of *H. pygmaea*.

M. barnesi is less common than *H. pygmaea* and also has a low reproductive potential, producing nineteen young in 8 days. It is not therefore such an important pest and population studies have been neglected.

Economic losses caused by cecids are most evident in these *Mycophila* species. The larvae are orange in colour, unlike the white Heteropezinae, and it is here that the main cause of economic loss lies. The larvae climb the stems of the mushrooms, often in vast numbers. Though minute, their vivid colour makes them conspicuous and the mushrooms are rendered unmarketable.

In one experiment where only ten *M. speyeri* larvae were introduced to 6 sq.ft. trays at spawning, 51 % of the mushroom crop was visibly infested with larvae, most damage being done early in the crop when the population was at its peak. *M. barnesi* caused most damage towards the end of cropping but nevertheless spoilt 57 % of the crop.

In *H. pygmaea* there is less tendency to climb on to the mushrooms and their white colour makes them less conspicuous. Thus although population growth was more rapid than in *M. barnesi* and final populations much higher, only 21 % of the total crop was visibly infested, most damage occurring in the last 3 weeks. However, *H. pygmaea* causes loss in yet another way. It is almost invariably associated with a disorder, known to growers as 'nibbling of the gills', in which the gills undergo bacterial disintegration. Also a disease known as 'bacterial pit', which affects the upper surface of the cap, is often prevalent when *H. pygmaea* is present. It is likely therefore that other bacterial diseases affecting the mycelium may be disseminated by this species, which may explain the death of mycelium and failure of crops so often attributed by growers to cecids. Bacterial disorders have never been encountered in association with the *Mycophila* species even when the two genera have been reared in adjacent trays in randomized experiments. This difference between the species has yet to be explained.

One is therefore faced with a pest with which it is very difficult to contend. A very small initial infestation early in the spawn-run can build up to a very large population by the time cropping starts. It is virtually impossible to detect and prevent the first ingress, and once established there is little hope of controlling the growing population. Attempts at control must therefore aim at prevention rather than cure. Incorporation of insecticides in the compost before filling the house has given promising results, but the chemicals decreased yield. As cecids do not affect fruiting, a new line of investigation is being followed, so far with good results. Insecticides are being mixed with the casing soil (a mixture of peat and chalk which is spread over the compost). Although larvae are able to reproduce within the compost, the treated casing forms a barrier which prevents the larvae from migrating upwards on to the mushrooms.

The problem seems to resolve itself, not into one of complete extermination of the pest, but of restricting its movements or its population to within economic limits. It would be sufficient for treatment to slow the rate of reproduction by a few days or decrease the size of family, rather than to kill, thus ensuring that a population would not grow to economic levels before the end of cropping. As population growth is exponential, small differences in reproductive potential can have profound effects on population growth.

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GROWTH OF THE POTATO PLANT

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The normal growing season of the potato is limited at both ends—by low temperatures and late frosts in the spring and by early frosts or blight in the late summer. Moreover, tuber growth generally occurs over a period of decreasing radiation and temperature. Higher yields may be expected if earlier tuber initiation could be encouraged, the period of tuber growth extended, and/or the rate of bulking increased. The influence of size of sprout at planting and temperature on these growth characteristics was explored.

Detailed observations on sprout growth during chitting showed a close curvilinear relationship between dry weight or number of leaves differentiated and length, irrespective of position on the tuber, temperature during chitting or variety. When tubers were planted in the field, those with very short sprouts emerged much later, showed a longer time between emergence and tuber initiation and had a slower rate of bulking than tubers with sprouts about 1·5 cm. long. Sprouts of greater length than this did not lead to earlier emergence or to earlier tuber initiation. There is therefore a definite limit to the advantage that can be obtained by preplanting growth; sprouts greater than 1·5–2 cm. long provide no advantage. There was a similar time to emergence and initiation from both Arran Pilot and King Edward provided the sprouts were of the same length; some of the differences between earliness of varieties may be therefore associated with sprouting behaviour during chitting (as well as rate of bulking).

Tubers at different stages of sprout development during chitting were planted in compost at constant depth and kept in a constant environment. The greater the size of sprout the earlier was emergence but tuber initiation was most rapid from sprouts of intermediate size. There was a slower relative growth rate after emergence with the larger sprouts over the range of sprout size examined. The reasons for this have not yet been uncovered; it seems likely that differences in number and size of meristems are associated. The influence of nutrient concentration in the external solution is also being explored and the amount of material transferred from the mother tuber to the growing plant calculated. Up to the time of development of a leaf surface of 200 cm.^{2}, most of the material in the sprout comes from the tuber; only 6 % was added by assimilation. This is a surprisingly small amount. Respiration accounted for 13 % of the losses from the parent tuber; these followed very closely the growth made by the sprout.

The influence of temperature was studied on Arran Pilot grown in pots in the greenhouse, in the field and in plant-growth rooms. Emergence was always earlier the higher was the temperature at planting, but high temperatures after emergence tended to delay tuber initiation. The earliest initiation and the greatest number of tubers were encouraged by low temperatures but after initiation tuber growth was fastest at a temperature of 15–20° C. These responses were closely associated with the differential growth of the various organs. High temperatures encouraged haulm growth and stolon growth, the long delay in tuber initiation being apparently associated with a low concentration of carbohydrates at the stolon tips. Previous workers have suggested that less assimilate is available for growth at high than low temperatures, because of excessive loss in respiration. This is not so; more assimilate was used in growth at 25° C. than at lower temperatures but, also, a higher proportion of it was diverted to the haulm. Those plants which initiated an intermediate number of tubers early in ontogeny showed the highest rates of bulking. A large number of tubers appeared to reduce the rate of bulking as well as giving a large number of small-sized tubers. The rate of bulking was also a positive function of incoming radiation and day temperature

and a negative function of night temperature, but was not closely associated with the leaf area duration or the net assimilation rate.

The differential effect of temperature suggested that planting at high temperatures followed by transplanting into the field would lead to earlier initiation. This was found to be so but the rate of bulking was reduced. The reasons for these responses are being explored in detail.

The length of the photoperiod was found to have very little effect in these experiments. It is suggested that most European varieties are day-neutral with a tendency to short-day reactions but that the response to daylength is so small relative to the responses to other factors, such as daily radiation and temperature, that it is of no practical or physiological interest.

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THE BOURSE SHOOT AS A FACTOR IN THE GROWTH OF APPLE FRUITS

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(With 2 Text-figures)

With increasing production of apples, quality and controlled cropping are becoming of paramount importance; much is already being done to cut down waste by the efficient use of fungicides and insecticides, but considerable losses still occur because of the production of undersized fruits. On the other hand very large dessert apples are not popular in this country and so the grower must always be striving to produce fruits of an optimum size. Over-cropping in any one year must be avoided as this tends to encourage biennial bearing with all its disadvantages; finally, the fruits must be of attractive appearance, good flavour and of suitable storage quality.

Fruit growth and development is obviously influenced by many factors, such as pollination, mineral nutrition and hormonal control by the seeds and these have been and are still being investigated, but other possible factors have not received so much attention. One such possible factor is the effect of the bourse shoot upon fruit growth. In the apple tree, flowers are usually produced on a short shoot or spur. Below the terminal cluster of flowers is a close spiral of leaves in the axil of each of which is a vegetative bud. These may remain dormant, or one or more may grow out to form a vegetative shoot known as a bourse shoot. If such a shoot grows out, does it compete with the fruitlets for food reserves or, as it later develops a considerable leaf area, does it contribute to the growth of the fruits by an additional supply of carbohydrates? The experiment to be outlined was designed to clarify this point.

Experience has shown the difficulty of carrying out such an experiment on orchard trees because of the interaction of branch systems, and the problem of the selection of uniform material. The experiments have therefore been made on small trees raised in pots as follows: fruiting wood of the variety is grafted in January or February to stocks already established in pots. These are placed in a cool greenhouse and one maiden shoot is allowed to grow from each graft. As terminal shoot growth slows down in July, the plants are transferred to a frame and the shoots tied down to horizontal wires to encourage fruit-bud formation. The trees are available for experimental use in the following year when they consist of a single horizontal shoot well furnished with fruit buds. The trees used in this experiment were, in fact, 2-year-old trees of the variety Lord Lambourne which had not been used in their first year.

In early March, as the buds were beginning to swell, a fruit bud approximately half way

along the shoot was selected and labelled. The shoots of all the trees, other than controls, were then cut back to encourage vegetative growth as follows:

Treatment 1 Control—shoot not cut back.
2 Bourse shoot allowed to grow from labelled spur.
3 Bourse shoot allowed to grow from spur next distal to labelled spur.
4 Bourse shoot allowed to grow from spur next proximal to labelled spur.
5 Shoot cut back, but no bourse shoot allowed to grow.

All other buds, spurs or shoots were removed or pinched out as soon as visible. The treatments are shown diagrammatically in Fig. 1. All flowers were removed other than from the labelled spurs and the spurs of the control trees, and those remaining were pollinated by brush with pollen from the variety Worcester Pearmain. There were five replicates per treatment. The results of treatments 3 and 4 were so similar that these have been regarded as a single treatment.

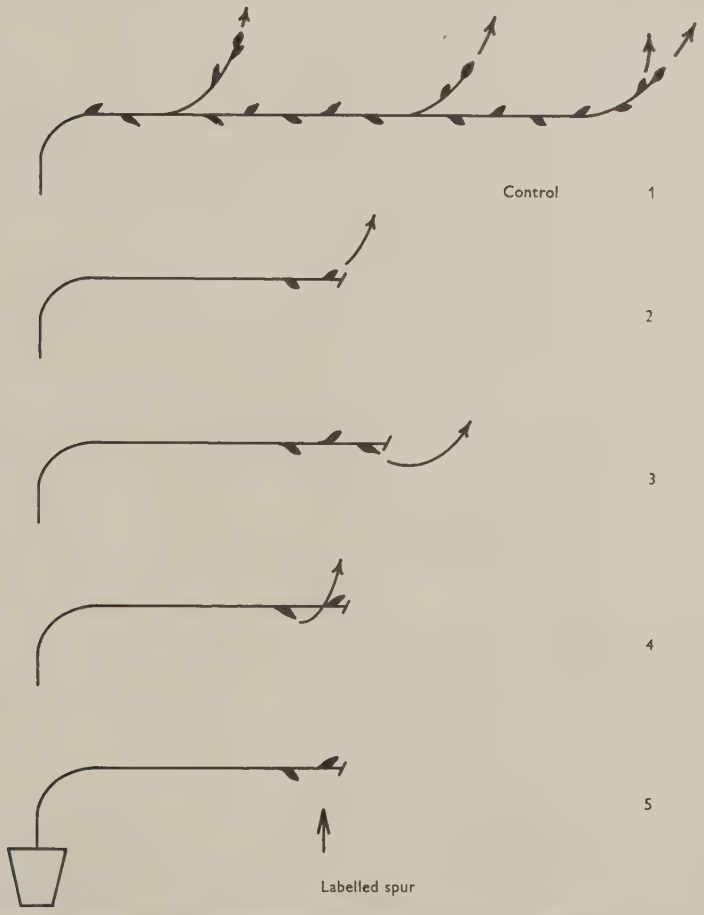

Fig. 1. Diagrammatic representation of treatments. For explanation see text.

Fruit diameters were measured at frequent intervals with calipers and growth curves were constructed from a mean of only those fruits reaching maturity.

In spite of adequate pollination all the fruitlets of the control trees abscissed in the 'first drop' (Fig. 2). Approximately 2 weeks later those of the other treatments began to fall, first those of treatment 2, then those of treatment 3 and 4 and finally those of treatment 5. The time of fruit drop was reflected in the extent of drop since all those in treatments 1 and 2 abscissed while the least drop occurred in treatment 5.

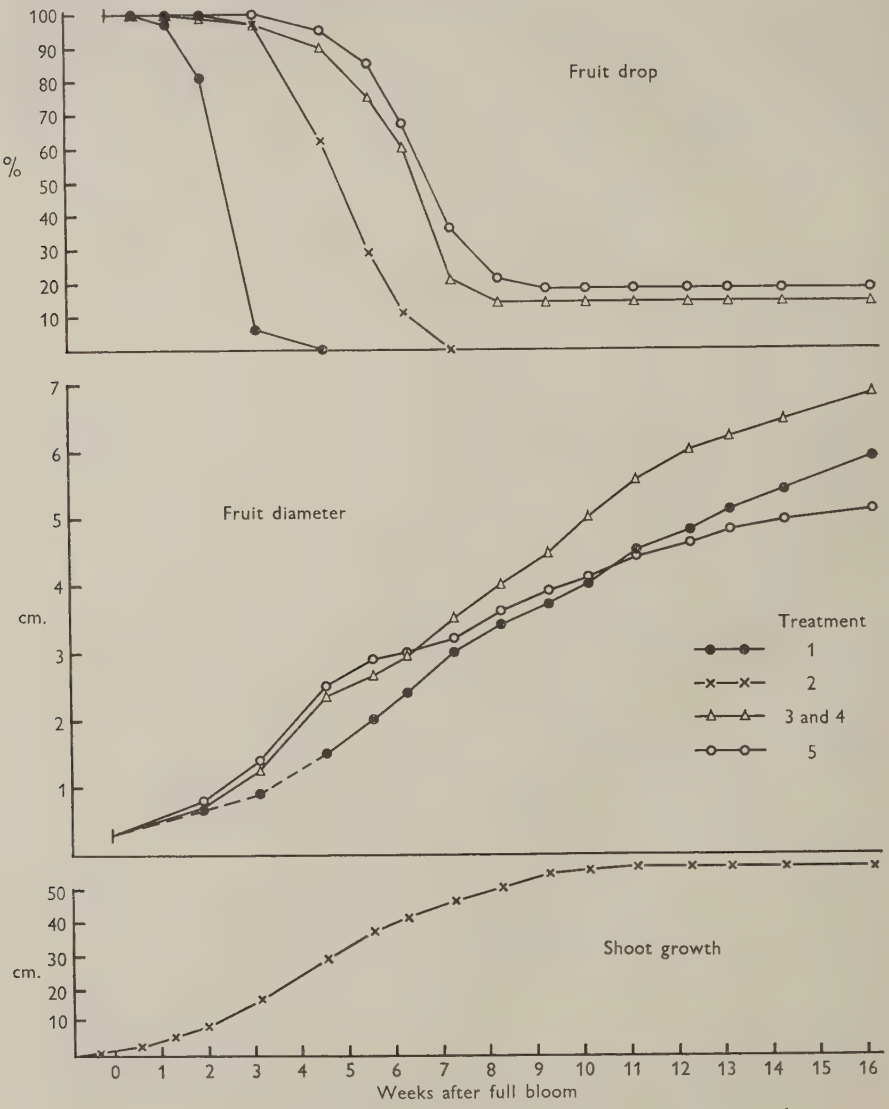

Fig. 2. Effect of treatments on fruit drop and diameter, and shoot growth.

No accurate growth curve is available for the control fruits for the first few weeks since, as already mentioned, all the fruits on the labelled spurs abscissed. When this became apparent, however, fruits on other spurs on the control trees were measured and the growth curve has been constructed from these. It is a fairly smooth, sigmoid type of curve typical of the growth of apple fruits. The effect of pruning on the other treatments has been to increase the rate of growth very considerably in the early stages of development, but this began to slow down after approximately 4 weeks after full bloom, and that of treatment 5, which showed the highest initial rate, continued at a low level until harvest. The growth rate of treatments 3 and 4, however, showed a renewed increase, and these fruits grew rapidly until harvest.

Shoot growth on the pruned treatments commenced just before full bloom, reached its maximum rate about $4\frac{1}{2}$ weeks after full bloom and ceased after another $5\frac{1}{2}$ weeks, when the mean length of the bourse shoots was 52 cm. There was no marked difference between the growth of the shoots on any of the pruned treatments, but total vegetative growth, both from bourse and terminal buds on the control trees was very much smaller, being only 19 cm. per tree when growth ceased.

As an experiment to investigate the influence of the bourse shoot on the growth of apples in the field, it might be said that the design leaves something to be desired, but the very fault, namely, the severe pruning to induce shoot growth, does itself produce some interesting results. It is noteworthy that no fruits persisted on the control trees while a 100 % initial set was obtained on the other treatments. This emphasizes the fact that pollination, however good, is by no means the determining factor in fruit set. It appears that an adequate supply of nutrients must be available from storage in the stem and/or roots. In the control trees these nutrients seem to be dissipated throughout the many centres of meristematic activity in the production of leaves, flowers, etc., and so are at a low level in any particular spur. In the pruned treatments they are channelled into only two or three clusters and are, therefore, available in sufficient quantities to allow not only fruit set, but also the growth of a vegetative shoot. As this shoot grows, it becomes a considerable sink for the food reserves and so competes with the fruit. All the fruits on treatment 2 absciss, but those on treatments 3 and 4 persist longer and some remain to harvest. The extent to which the shoot competes with the fruit may be associated with the intimacy of their vascular 'supply lines' for, in treatment 2 these must be closely connected and must exploit the same food reserves, whereas in treatments 3 and 4, because of the phyllotaxis of the shoot, the spurs are on different segments and therefore not in such severe competition. The last and fewest to absciss are those fruits of treatment 5 which begin to drop as their reserves are exhausted.

At about this time the bourse shoot begins to contribute nutrients to the fruit and the growth of the apples in treatments 3 and 4 begins to increase and overtake that in treatment 5. After the poor initial start on the control trees, the many leaves gradually contribute and the growth of fruits on these trees continues steadily, also eventually overtaking those in treatment 5 which have only very few leaves.

This interpretation in terms of competition for nutrients may seem rather naïve in these modern days of growth substances. It might be argued that the plant parts distal to the labelled spur are producing a supra-optimal level of auxin or some inhibitor which slows down the rate of growth of the fruits or causes them to absciss; but then why do those fruits of treatment 5 slow down and absciss when there is nothing distal to them? Surely this must be due to lack of nutrients and, if this is so, why not apply the same explanation to the other treatments. Certainly auxins might be involved as being the mechanism whereby the nutrients are attracted to the sites of utilization, and nutrients must necessarily be a vague term including any growth factors such as a caline or gibberellin, since some such similar substance rather than a macro-nutrient may be the substance necessary to growth.

Indeed an anatomical examination of the fruits showed that apples from treatment 1 contained of the order of 12×10^6 cells/fruit, while those from treatments 3 and 4 contained 32×10^6 and those of treatment 5 contained 30×10^6 cells/fruit. It is known that cell division occurs in the fruitlet only during the first 3 weeks after full bloom and that further growth is by cell expansion only. This suggests that stored reserves available within the first few

weeks after full bloom are conducive to rapid cell division, while a plentiful supply of currently produced carbohydrate enables cell expansion to take place, for the mean cortical cell sizes of the fruits from treatments 1, 3 and 4, and 5 were 0·0094, 0·0066 and 0·0027 mm.^{3}, respectively. In other words, the amount of available stored reserves determines the potential size of the fruits, while the subsequent leaf area and thereby the carbohydrate supply determines the extent to which the potential size will be realized. As might be expected, seed number appears also to be correlated with the availability of stored reserves, since the control trees had a mean seed number of 2·7 per fruit, treatments 3 and 4 had 6·0 and treatment 5 had 6·5 seeds per fruit. There was, however, no difference between the weights of the embryos in the various treatments.

The foregoing discussion is rather speculative and some of the suggestions made need confirmation, but it can be concluded that while a bourse shoot growing out from a fruiting spur probably competes with the fruit in the early stages of growth, it later ensures a high level of carbohydrates when the cells are enlarging. A bourse shoot growing from a non-fruiting spur similarly benefits the fruit on any adjacent spur. Thus the bourse shoot is not only a factor in controlling the size of the fruit, but, by influencing the composition of the fruit, may also affect its storage quality.

Ann. appl. Biol. **48** (2), 439–441.

REVIEWS

Grasslands (A.A.A.S. Symposium). Edited by H. B. Sprague. Pp. 424. Publication no. 53 of the A.A.A.S.; agents: Bailey Bros. and Swinfen, London. 1959. 82*s*.

This volume comprises thirty-seven papers presented in 1956 at eight symposium sessions of Section O (Agriculture) of the American Association for the Advancement of Science. It would be invidious in a short notice to select any particular papers for mention: all are of a high standard and most point to gaps in our knowledge and the research needed to fill them.

Section I, 'Sciences in support of grassland research', well illustrates the variety of 'fundamental' sciences concerned and ways in which many of them are (or should be) integrated. The second section, 'Forage production in temperate humid regions', assesses the position and contribution of grasslands in the agriculture of the U.S.A., and also describes plant breeding and pest and disease control. In 'Engineering aspects of grassland agriculture', the authors present a summary of techniques and equipment available for use at all stages of grassland production, from sowing, establishment and irrigation to feeding of preserved material; without turning the section into the catalogue it might easily have become.

Sections IV and V, 'Forage utilization and related animal nutrition problems' and 'Evaluation of the nutritive significance of forages', are concerned with the end-products of grassland—animals. Most attention is given to dairy cows; a little to sheep. Sections VI, 'Grassland climatology', and VII, 'Ecology of grasslands', are connected by the ecological importance of climate, while the final section, 'Range management', contains papers on plant breeding and testing and on most aspects of ecological research on the range and prairie types of grassland, which cover 728 million acres—40 % of the U.S.A.

The book is clearly printed on good quality paper, and is well and attractively bound.

R. DORRINGTON WILLIAMS

Symposia of the Society for Experimental Biology. XIII. Utilization of Nitrogen and its Compounds by Plants. Edited by H. K. Porter. Pp. 385, 5 plates. Cambridge University Press. 1959. 50*s*.

The present volume in the series contains the texts of the papers given at a symposium held at Reading in September 1958. The book is well edited and produced; the time lag between its appearance and the meeting was commendably short. The symposium was a small-scale international congress and of the twenty papers presented half were by conference members from overseas. As is usual in a symposium of this kind some papers mainly reviewed work already published whereas others described previously unpublished results. A wide range of topics is covered and various aspects of nitrogen metabolism in yeasts, algae, fungi and higher plants are discussed. Many of the papers contain a great deal of detailed information and include some complicated chemistry; they cannot therefore be recommended as light reading.

Four papers are concerned with different aspects of nitrogen fixation. Roberts discusses the chemistry of the process and puts forward several plausible reaction schemes involving reduction, oxidation, direct reaction with organic substances and hydrolysis. Nutman considers the various reasons why the symbiosis in the nodules of *Trifolium* roots may not function effectively. The normal development of symbiosis can be blocked at all stages of the process. There may be failure of infection of the root hairs; intracellular invasion and bacterial multiplication may be inhibited and lastly the nodule tissue may degenerate prematurely. The biochemistry of haem compounds found in legume root nodules is

described by Falk, Appleby and Porra. Their study was made to investigate the initial steps which bring atmospheric nitrogen into a reactive state and also the biochemical basis of effectiveness in symbiosis. They found that ineffective nodules possessed the necessary enzymes for haem synthesis, but lacked an essential heat-stable factor present in effective nodules and were therefore unable to synthesize the porphyrins as could effective nodules. Bond reviews nitrogen fixation in root nodules of plants which are not members of the Leguminosae. The group considered comprises eight genera from five families and all are either trees or shrubs.

Several papers deal with the occurrence and role of amino acids. Fowden gives an account of non-protein amino acids, most of which have only been discovered since chromatographic methods have been applied. Synge discusses non-protein chemically bound forms of amino acids with particular emphasis on the analytical techniques available for recognizing them. In the past these compounds have usually been reported as 'peptide N' and little has been done to characterize them. Although they occur in low concentration they may be of considerable biochemical importance. Miettinen presents results obtained by the use of isotopes which show that amino acids can be taken up, without decomposition, by pea roots. In a paper on the metabolism of aspartic acid, serine and glycine in higher plants Davies, Hanford and Wilkinson 'set up heuristic hypotheses' and discuss them in the light of available evidence. The interrelations of ornithine, citrulline and arginine are described by Naylor. Even though only a few higher plants have so far been studied the metabolism of these compounds seems to be as variable as in animals, bacteria and fungi. Folkes compares the position of amino acids in the assimilation of nitrogen and the synthesis of proteins in a yeast (*Torula*) and a green plant (Barley). The biosynthesis of amide and peptide bonds with special reference to the formation of glutamine by glutamine synthetase is reviewed by Webster. He also gives some account of the 'activation' of amino acids, one of the probable steps in the synthesis of protein.

A long paper by Steward and his associates is concerned with the effect on nitrogen metabolism in higher plants of factors such as growth under long and short days, high and low night temperatures and their interaction with inorganic nutrition. A complicated picture results from such experiments and special interpretative techniques are required to understand the interplay of so many variables.

Nicholas describes the reduction of nitrate to ammonia in *Neurospora* by a series of reactions, each of which is catalysed by a metallo-flavoprotein enzyme. Nitrate reduction in green algae, discussed by Kessler and Fogg, deals with the special case of nitrogen nutrition and metabolic patterns in unicellular algae. Steiner discusses the utilization of amide and amino nitrogen by yeasts with particular reference to metabolic reactions at the surface of the cells.

The increasing use of urea as a source of nitrogen applied to the soil or the aerial parts of plants has aroused interest in its utilization. Bollard discusses this and also the occurrence of compounds which can yield urea or which can be formed from it.

Heyes describes changes that occur in nucleic acids during cell growth and development and their role in the mechanism of protein synthesis in cultured pea roots.

Mothes discusses the biosynthesis of alkaloids and Sokolov considers some of the environmental factors which influence the formation of these compounds, many of which are of pharmacological importance.

MARGARET HOLDEN

Biochemistry of Insects. Ed. L. Levenbrook. (Proceedings of the Fourth International Congress of Biochemistry. Vol. xii, Symposium xii.) Pp. xviii + 252. London: Pergamon Press. 1959. 70*s*.

This volume contains the text of fourteen papers given to the Symposium on the Biochemistry of Insects at the Fourth International Congress of Biochemistry, held in Vienna in 1958. Eleven other symposia on such subjects as carbohydrates, proteins, viruses and vitamins

also took place, and the whole congress has been reported in a series of fifteen volumes (of which this is one) costing a total of £48.

The Insect Biochemistry Symposium dealt with certain topics, such as the two papers on how insecticides affect insect metabolism, which are of practical interest to economic entomologists. The paper on host specificity of phytophagous insects is also of economic importance, though it is really on plant rather than on insect biochemistry. Several papers, including those on the cuticle, on insect poisons and on silkworms, concern the entomologist with biochemical interests. Many of the remaining papers, valuable contributions in their fields, show that insects are useful experimental animals to elucidate biochemical problems common to other groups. This was perhaps the most important contribution of the whole symposium.

It is suggested that this symposium is a recognition that 'insect biochemistry' is now a specialist field, equivalent in standing to those covered by the other symposia. This is questionable. Although most of the papers will be of some interest to a wide range of entomologists, an entomological biochemist specializing in the study of lipoids (for instance) may have more in common with a colleague working on the fats in vertebrates than with an entomologist concerned with hormones or vitamins.

This is the report of an international conference, and is published partly in English and partly in German. The opening remarks of Professor Butenandt are in German, with a full English translation. Thirteen of the fourteen papers are in English, with no translation, even of an abstract, in any other language. The single paper in German has a one-page English abstract. The rather scrappy reports of the discussions are in the language in which the contributions were spoken, either English or German, with no translation. This all means that more than 90% of the text is in English, and unless the reader understands that language the volume will be useless to him. The small amount of German can only serve as an irritation to the monoglot English or American reader.

Several papers have long bibliographies which might have been useful had the authors been listed in alphabetical order; they have been made almost valueless by being printed in the order in which they appear in the preceding paper. There is no index.

K. MELLANBY

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1959

The Officers and other Members of the Council of the Association were as follows:

President: R. V. Harris, D.Sc., A.R.C.Sc., M.I.Biol.
Vice-Presidents: Miss M. D. Glynne, D.Sc., M.I.Biol.; C. Potter, D.Sc., Ph.D., D.I.C., M.I. Biol. (*President-elect*).
Treasurer: H. F. Barnes, M.A., Ph.D., M.I.Biol.
General Secretary: L. Broadbent, D.Sc., Ph.D., M.I.Biol.
Programme Secretary: W. G. Keyworth, A.R.C.Sc., Ph.D., M.I.Biol.
Editor: R. W. Marsh, M.A., M.I.Biol.
Assistant Editor: I. Thomas, M.Sc., Ph.D., M.I.Biol.
Other Members: P. Aitkenhead, B.Sc.; C. C. V. Batts, B.Sc., Ph.D.; A. E. W. Boyd, B.Sc., Ph.D.; A. H. Bunting, M.Sc., D.Phil., M.I.Biol.; J. F. H. Cronshey, M.A.; Miss M. T. Franklin, B.Sc., Ph.D., M.I.Biol.; J. Hobart, B.Sc.; A. H. M. Kirby, M.Sc., Ph.D., A.R.I.C.; F. Raw, B.Sc., Ph.D., M.I.Biol.; M. E. Solomon, M.Sc., M.I.Biol.; M. J. Way, M.A., M.I.Biol.; J. H. Western, B.Sc., Ph.D., M.I.Biol.

Thirty-two new Ordinary Members were elected during 1959, and one resignation was accepted during the year. Membership was 749 on 31 December 1959, 177 being resident overseas, but 10 Members resigned at the end of the year. Seven others ceased to be members in accordance with Law VII. Three Members were elected as from 1 January 1960 on which day the Membership was thus 735 (701 Ordinary Members, 13 Honorary and 21 Life Members), an increase of 14 over the previous year.

Honorary Membership of the Association was conferred on W. Buddin, M.A., formerly Advisory Plant Pathologist, N.A.A.S., distinguished for his work on disease assessment and for stimulating research on new plant pathological problems, who joined the Association in 1920 and was a Councillor during 1932–4: and on F. Tattersfield, O.B.E., D.Sc.,F.R.I.C., formerly Head of the Department of Insecticides and Fungicides, Rothamsted Experimental Station, distinguished for his research work on insecticides, particularly those derived from plants, and on the quantitative biological assessment of insecticidal action, and as one of the founders of the pyrethrum industry in Kenya. He joined the Association in 1921.

The Council was grieved to hear of the deaths of the following Members: Prof. Dr J. H. Schuurmans Stekhoven, of Utrecht, Netherlands, on 11 November 1958; Dr F. Tattersfield on 1 May 1959; Miss E. I. MacGill on 22 September 1959.

Dr Frederick Tattersfield, O.B.E., D.Sc., F.R.I.C., died on 1 May 1959 aged 78. He was a leading authority on insecticides and founded the Department of Insecticides and Fungicides at Rothamsted Experimental Station in 1917 when he was invalided out of the Friends' Ambulance Unit. Dr Tattersfield's researches covered a wide field including insecticides for use in soil and on the aerial parts of plants. He discovered the outstanding ovicidal properties of dinitro-*o*-cresol, a substance which has been used as a winter wash on fruit trees ever since. Dr Tattersfield contributed largely to our knowledge of the insecticides derived from plants, particularly derris and pyrethrum, and played a considerable part in founding the Kenya pyrethrum industry. Also he was the first to develop adequate quantitative methods of laboratory bioassay.

Dr Tattersfield set himself very high standards in his own work and by his example he raised the level throughout his subject. He was always deeply interested in the Association, which by his death has lost a most valued member.

Miss E. I. MacGill, M.Sc., a Member since 1929, was appointed Assistant Lecturer in Zoology in the University of Edinburgh after graduating at Manchester. After 3 years she

returned to the University of Manchester and spent the rest of her professional life there in the Department of Zoology, from 1925 to 1948 as a Research Assistant in Entomology on a grant from the Empire Cotton Growing Corporation. From 1948 to 1958 she was part-time Demonstrator in Zoology and for the final year was Curator in the Department. She published an interesting series of papers on the cotton stainer and *Thrips tabaci*.

The Council noted with pleasure the conferment of the honour of O.B.E. on Dr E. K. Woodford, and the election to fellowship of the Royal Society of Prof. G. E. Blackman and Prof. O. W. Richards.

The Council met seven times during 1959. Among matters discussed were the reproduction of early volumes of the *Annals* and the extension of the Association's activities to ensure adequate coverage of rapidly developing new fields in applied biology. It was decided to increase the number of general meetings per year so that one or two could be devoted to progress reports covering a wide field in a particular subject.

The Association was represented on the Parliamentary and Scientific Committee by the General Secretary, Biological Council by the General Secretary, National Committee for Biology (Royal Society) by the General and Programme Secretaries, British Weed Control Council by Dr E. K. Woodford and the British Standards Institution Technical Committee for Nomenclature for Pesticides by Dr J. R. Busvine.

The Council thanks the Governing Body of Imperial College, and the Trustees of the British Museum for their kindness in providing lecture theatres for General Meetings of the Association; also the Agricultural Research Council for kindly providing meeting rooms for the Council.

Four paper-reading meetings were held in London, with an average attendance of 60 Members and 46 Visitors. Papers read at these meetings were as follows:

9 *January*. Symposium on 'Insects and public health'. J. KEIDING: Housefly control and resistance to insecticides in Danish farms. J. R. BUSVINE: Insecticide resistance in bed bugs. R. J. WOOD: Insecticide resistance in mosquitoes. D. S. KETTLE and R. H. PARISH: The control of biting midges in Scotland. B. C. HOBBS: The significance of insects in the contamination of foods.

6 *March*. Symposium on 'Recent advances in plant propagation'. G. K. ARGLES: The effects of timing and of environment on root formation by cuttings. F. H. VANSTONE: Equipment for mist propagation developed at the N.I.A.E. J. T. SYKES and I. H. WILLIAMS: Some factors affecting regeneration of cuttings under mist technique. C. D. DEMPSTER: Propagation of evergreen shrubs and conifers under double glass and mist. E. S. J. HATCHER: The propagation of fruit tree rootstocks from stem cuttings. F. W. SHEPHERD: A comparison of methods of striking softwood cuttings of *Cupressocyparis leylandii*, Escallonia 'Crimson Spire' and *Pittosporum tenuifolium*. Miss E. EMMERSON: Investigations into the vegetative propagation of Pelargoniums.

23 *October*. Symposium on 'Control of harmful birds'. H. V. THOMPSON: Economic ornithology. C. J. DUNCAN: The sense of taste in birds. R. J. P. THEARLE: The use of narcotics in catching harmful birds. E. N. WRIGHT and D. D. B. SUMMERS: Biology of the bullfinch and its economic importance. R. K. MURTON: The population dynamics of the wood-pigeon and methods of control.

4 *December*. I. J. WYATT: Cecidomyiidae as pests of cultivated mushrooms. G. A. WHEATLEY and J. A. HARDMAN: The bio-assay of residues of insecticides in soil. M. ALLEN: The uptake of magnesium from applications to leaves of apple trees. D. G. GANDY: A transmissible disease of cultivated mushrooms ('watery stipe'). J. E. CROSSE: Fire-blight of pear in Britain. H. H. GLASSCOCK: Fire blight: history in Britain.

17 *April*. 47 Members attended the Annual General Meeting. Later 6 Visitors joined the Members to hear the Presidential Address by Dr I. Thomas on 'Control and conservation'.

22 *May*. About 50 Members and their guests visited the Forest Products Research Laboratory (D.S.I.R.), Princes Risborough, Bucks. Council wishes to thank the Director, Dr Henderson, and his staff for a most interesting and enjoyable visit.

17–18 *September*. The Provincial Meeting was held in the University of Exeter. 35 Members and 8 Visitors were welcomed by the Deputy Vice-Chancellor and Professor of Botany, Dr J. Caldwell, after which the following papers were read: J. WILKINSON: The effects of virus on nuclear division. J. E. E. JENKINS: Bronzing of tomato fruits. G. J. LINN: Estimation of populations of small mammals. H. C. WOODVILLE: Some entomological problems in South-West England.

During the afternoon of the 17th the party visited the N.A.A.S. South-West Regional Sub-centre at Starcross. Some of the advisory and investigational work being done by the Entomology, Plant Pathology and Bacteriology Departments was described and several very interesting exhibits had been arranged.

During the evening Prof. Caldwell conducted the party around the campus, particularly the gardens, and then acted as host at a University reception.

On 18 September the following papers were read: G. C. AINSWORTH: Recent trends in studies on fungous diseases of man and animals. W. CARRUTHERS: Carcinogens in petroleum and tobacco smoke. J. G. JENKINSON: Scorch of broccoli.

Exhibits were arranged by Miss S. M. Robb on virus diseases of plants and Mr D. E. Eveleigh on fungi attacking painted surfaces. During the afternoon the Seale-Hayne Agricultural College, Newton Abbot, was visited. After an account of the College's activities by the Vice-Principal, the buildings, Farm and N.I.A.B. Trials were inspected. The meeting ended with tea, generously provided by the College.

The Council wishes to thank the Governing Body of Exeter University and the Staffs of the biological departments, the N.A.A.S. Staff at Starcross, and the Principal and Staff of Seale-Hayne Agricultural College for their generous hospitality and co-operation in making this a most enjoyable and instructive meeting; and also Dr S. A. J. Tarr, the local secretary, and Miss S. M. Robb who assisted him, for arranging the meeting and the excellent accommodation at Lopes Hall.

The following are the addresses of non-Members whose papers (listed above) have not been printed in Proceedings;

M. Allen, East Malling Research Station; W. Carruthers, Cancer Research Department, University of Exeter; D. S. Kettle and R. H. Parish, Department of Zoology, University of Edinburgh; G. J. Linn, Department of Zoology, University of Exeter; J. Wilkinson, Department of Botany, University of Exeter.

REPORT OF THE HONORARY EDITOR FOR 1959

Volume **47**, of 811 pages, contained 79 papers (of which 13 were Proceedings papers),with 16 plates. The previous volume, of 678 pages with 19 plates, contained 72 papers. A comparison might also be made with the 1939 volume which on 849 pages carried only 59 papers, of which 18 were Proceedings papers.

A rough grouping of the subjects of the papers in Volume **47**—with Proceedings papers in parentheses—gives: Entomology and insect pests, 14 (2); Mycology and fungus diseases, 18 (2); Bacterial diseases, 8 (1); Viruses and virus diseases, 8; Insecticides and fungicides, 7 (2); Applied plant physiology, 14 (5); Nematology, 5 (1); Others, 5. The increased number of papers in applied plant physiology is noteworthy.

The printing stoppage in the summer delayed the issues of nos. 2, 3 and 4 which appeared, respectively, on 16 July, 9 December and 27 January.

Work on the fifty-year Index is proceeding satisfactorily. Preliminary drafts have been sent to members of the Publications Committee and others interested, who have made valuable comments. For this, in addition to their many other services, the help of the Publications Committee is gratefully acknowledged.

REPORT OF THE ACTING HONORARY TREASURER FOR THE YEAR ENDING 31 DECEMBER 1959

Income from the sale of the current volume (Vol. **47**) of the *Annals of Applied Biology* was £3363, an increase of £54 over sales of Vol. **46** during 1958. Income from back volumes and parts was £347, an increase of £31 over corresponding sales in 1958. Income from reprints and advertisements was £257, a decrease of £150 on corresponding sales in 1958. Volume **47** cost £5671 to produce with a net cost to the Association of £1820, as compared with £923 in 1958. During 1959, £1178 was received in current subscriptions and £26 in arrears, an increase of £8 and £11 over corresponding items for 1958.

There was a deficit of £656 on the year's working as compared with a profit of £186 in 1958.

The net assets of the Association at the end of 1959 were £4532, as compared with £5188 on 31 December 1958. All the available surplus funds are invested in National Savings Certificates, 4½% Defence Bonds and 5% Defence Bonds and on deposit at the Post Office and in the Westminster Bank. The estimated value of the stock of the *Annals of Applied Biology* is £258.

In presenting this Report it is fitting to record the great indebtedness of the Association to its late Treasurer Dr H. F. Barnes. The accounts for 1959 were prepared by him for audit shortly before his death. The acting Treasurer gratefully acknowledges the valuable help which the auditor has given him since Dr Barnes's sudden death.

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1959 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To Estimated value of Stock, 1 January 1959		290 16 0	By Sales—Current volume	3362 12 3	
To Cambridge University Press:			Back volumes, parts and sets	347 8 2	
Paper and Printing	3778 0 8		Reprints and Advertisements	257 9 6	
Binding	355 4 4				3967 9 11
Carriage	284 11 5		By Estimated value of Stock—31 December 1959		258 0 0
Insurance and Sundries	107 2 10		By Balance carried down		1820 7 11
Commissions	1145 16 2				
		5670 15 5			
To Preparation of Index Vols. 1–50		84 6 5			
		£6045 17 10			£6045 17 10

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1959 *Cr.*

Expenditure	£ s. s.	£ s. d.	Income	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		1820 7 11	By Members' Subscriptions:		
To Printing and Stationery	109 13 3		Arrears	26 10 0	
To Postages and cheque stamps	60 5 1		Current	1177 17 6	
To Subscriptions and Donations:					1204 7 6
Parliamentary and Scientific Committee	7 7 0		By *List of Common Names*		10 0
Biological Council	1 15 0		By Interest received and receivable		
To Sundry out-of-pocket expenses of Editors, Secretaries and Treasurer	12 17 3		5% Defence Bonds	100 0 0	
To Institute of Biology—Fee for Secretarial Services	15 0 0		4½% Defence Bonds	45 0 0	
To Refreshments and Hire of Rooms	45 15 8		National Savings Certificates	71 2 11	
To Audit Fee	15 15 0		Post Office Savings Bank	5 9 0	
		268 8 3	Westminster Bank—Deposit Account	6 6 10	
					227 18 9
			By Balance being excess of Expenditure over Income for the year		655 19 11
		£2088 16 2			£2088 16 2

Dr. BALANCE SHEET AT 31 DECEMBER 1959 *Cr.*

Liabilities and Surplus	£ s. d.	£ s. d.	Assets	£ s. d.	£ s. d.
Sundry Creditors and accrued charges		1839 10 6	Balances at Bank:		
Subscriptions paid in advance		55 11 9	Westminster Bank: Current Account	435 0 8	
Life Membership Fund Reserve:			Deposit Account	199 11 0	
As at 1 January 1959	356 0 0		Post Office Savings Bank Account	273 13 9	
Addition during year	23 0 0				908 5 5
		379 0 0	2600 National Savings Certificates (Cost £1715 1s. 0d.)		2664 15 10
Overseas Speakers' Fund			5% Defence Bonds		2000 0 0
As at 1 January 1959	50 0 0		4½% Defence Bonds		1000 0 0
Expended during year	25 0 0		Stock of *Annals of Applied Biology* as estimated		258 0 0
		25 0 0			
Excess of Assets over Liabilities:					
As at 1 January 1959	5187 18 11				
Less Excess of Expenditure over Income for the year 1959	655 19 11				
		4531 19 0			
		£6831 1 3			£6831 1 3

F. RAW, *Acting Hon. Treasurer*

I certify that the foregoing accounts are properly drawn up in accordance with the books, vouchers and documents produced to me, and in my opinion they exhibit a true and correct view of the state of the Association's affairs.

31 March 1960 (Signed) J. B. BENNETT *Chartered Accountant* } *Auditor*

CORRIGENDA AND ADDENDA TO LIST OF MEMBERS OF THE ASSOCIATION OF APPLIED BIOLOGISTS†

INCLUDING CHANGES OF ADDRESS

(*As notified to Hon. Secretary and Hon. Treasurer*)

HONORARY MEMBERS

1920 SMITH, K. M., C.B.E., D.Sc., Ph.D., F.R.S., A.R.C. Virus Research Unit, Huntingdon Road, Cambridge. (Hon. Mem. 1960.)
1960 WESTERDIJK, Prof. Dr JOHANA, Javalaan 20, Baarn, Netherlands (Hon. Mem. 1960.)

ORDINARY MEMBERS (*Life Members are marked**)

1951 AITKENHEAD, P., B.Sc., Ministry of Agriculture, Fisheries and Food Plant Pathology Laboratory, Hatching Green, Harpenden, Herts. (Council 1957–60.)
1958 ALI, M. A., B.Sc., M.A., Zaklad Zoologii Systematycznej, Uniwersytet M.C. Sklodowskiej, Lublin, ul. Glowackiego 2, Poland.
1959 ALLEN, M., B.Sc., East Malling Research Station, nr. Maidstone, Kent.
1946 ALLERTON, F. W., B.Sc., A.R.I.C., 29 Hillside Avenue, Woodford Green, Essex.
1952 ASHWORTH, R. DE B., M.Sc., Ph.D., A.R.I.C., Ministry of Agriculture, Fisheries and Food Plant Pathology Laboratory, Hatching Green, Harpenden, Herts.
1959 A'BROOK, J., B.Sc., A.R.C.S., Dip.Ag.Sci., D.T.A., Ministry of Agriculture, Mokwa, Northern Nigeria.
1959 BARLOW, H. W. B., B.Sc., M.I.Biol., East Malling Research Station, nr Maidstone, Kent.
1960 BAXTER, A. MANTON, Baxter and Guion Ltd., Museum Buildings, Priestgate, Peterborough.
1960 BEDDING, A. J., M.Sc., N.D.H., N.A.A.S., Fairfield Experimental Horticulture Station, Greenhalgh, Kirkham, Preston, Lancs.
1949 BERENDT, R. A., B.Sc., c/o Shell Chemical (Australia) Pty Ltd., P.O. Box 1713P, 278 Queen Street, Melbourne C.I., Australia.
1943 BOYD, A. E. W., B.Sc., Ph.D., The Edinburgh School of Agriculture, West Mains Road, Edinburgh 9. (Council 1959– .)
1942 BRIAN, P. W., M.A., Sc.D., Ph.D., F.R.S., M.I.Biol., Akers Research Laboratories, The Frythe, Welwyn, Herts. (President-Elect 1960; Council 1952–4.)
1955 BROWN, W. B., M.Sc., A.R.C.Sc., D.I.C., F.R.I.C., Pest Infestation Laboratory, London Road, Slough, Bucks.
1955 BULLOCK, J. A., B.Sc., A.R.C.Sc., 43 Leyborne Park, Kew Gardens, Richmond, Surrey.
1943 BUNKER, H. J., M.A., M.I.Biol., 17 Radnor Road, Twickenham, Middlesex.
1959 BURDEKIN, D. A., B.A., Dip.Ag.Sci., East Malling Research Station, nr Maidstone, Kent.
1956 BURGES, D. H., B.Sc., Ph.D., Pest Infestation Laboratory, London Road, Slough, Bucks.
1958 CAMMACK, R. H., B.Sc., Ph.D., Ministry of Agriculture, Fisheries and Food Plant Pathology Laboratory, Hatching Green, Harpenden, Herts.
1951 CAMPBELL, W., B.Sc., N.A.A.S., Trawscoed, Aberystwyth, Cards.
1959 CHADWICK, P. R., M.A., Pyrethrum Board of Kenya, Box 420, Nakuru, Kenya.

† See *Ann. appl. Biol.* **46**, 274, and **47**, 385.

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